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ERRATA

VOLUME I

Page 163, line 6 from foot of page, "CO_3" should read "SO_3".

Page 392, Table VI, heading of first column, "Incr. 1 c.c. over 2.0 c.c." should read "Incr. 1 c.c. over 0.2 c.c."; heading of second column "Incr. 1 c.c. over 1.0 c.c." should read "Incr. 5 c.c. over 1 c.c."; heading of third column "Incr. .5 c.c. over 0.2 c.c." should read "Incr. 1 c.c. over 0.2 c.c."

Page 505-508. "A Rapid Method for the Estimation of Calcium Oxide in Peat Soils," by R. A. Gortner. For index references, see Index of Vol. II.

Page 541, line 11 from bottom, "Oudenmans" should read "Oudemans."

Page 571, lines 6 and 7. "The effect of reaction on ammonification by these fungi is more pronounced in clay than in sandy soil" should read "The effect of reaction on ammonification by these fungi is more pronounced in sandy than in clay soil."

VOLUME II

Page 2, line 15, "Oudmann" should read "Oudemans."

Page 63, reference 9, "Keimghabt" should read "Keimghalt."

Page 64, reference 13, "Denitrifikateonsbakterien" should read "Denitrifikationsbakterien."

Page 102, legend for Plate I, figure 2, "or" should read "on."

Page following page 102, legend for Plate II, figure 2, "bed on right" should read "bed on left," and "bed on left" should read "bed on right."

Page 112, line 2, "48 per cent of iron" should read "48 per cent of Fe_2O_3".

Page 112, line 22, "indentification" should read "identification."

Page 118, line 2 in table, *"Peiicillium atramentasum"* should read *"Penicillium atramentosum."*

Page 118, line 17 from foot of table, *"Dematuim pallulans"* should read *"Dematium pullulans."*

Page 129, line 15 from foot of page, "Tukal" should read "Zukal."

Page 131, line 10, "me-licum" should read "medium."

Page 135, line 13 from foot of page, *"ciunobarinus"* should read *"cinnabarinus."*

Page 137, line 22, *"pollulans"* should read *"pullulans."*

Page 144, line 3 from foot of table, *"rozzum"* should read *"roseum."*

Page 144, last line in table, *"pollulans"* should read *"pullulans."*

Page following page 156, legend for Plate IV, fig. 3, "canidiophores" should read "conidiophores."

Page following page 156, legend for Plate V, fig. 11, "Fuckii" should read "Fuckelii."

Page 167, line 12, "hard" should read "hand."

Page 217, line 3, "3.0" should read "3.5."

Page 217, figure 1, "atmosphe es" should read "atmospheres."

Page 221, line 24, "1 tenth" should read "2 tenths."

Page 221, line 25, "2 tenths" should read "1 tenth."

Page 225, figure 2, "calci m" should read "calcium."

Page 230, figure 4, lettering across foot of cut should read: upper line, "R, R 2, R 3, R 4, R 5, R 6, R 7, R 8." and lower line "C 1, C 2, C 3, C 4, C 5, C 6, C 7, C 8, C 1, C 2, C 3, C 4, C 5, C 6, C 7, C 1, C 2, C 3, C 4, C 5, C 6, C 1, C 2, C 3, C 4, C 5, C 1, C 2, C 3, C 4, C 1, C 2, C 3, C 1, C 2, C 1.

Page 245, footnote, next to last line, "MgO" should read "Mg."

Page 248, line 28, insert "ratio" after "calcium-magnesium"

Page 252, reference 28, "Oesterhout" should read "Osterhout."

Page 258, line 17, *"Bacillus Rutida"* should read *"Bacillus Putida."*

Page 265, add footnote: "Per cent acidities are represented in terms of normal, rather than concentrated acid."

Page 266, line 11, "Stömer" should read "Störmer."

Page 271, figure 3, legend, add "(Series 2)."

Page 338, figure 6, legend, "CaO_3" should read "$CaCO_3$".

Page 411, Table VII, third entry, "Carbonate CO" should read "Carbonate CO_2".

Page 437, footnote, line 19, "sols" should read "soils."

Page 481, line 25, equation (2) "a" should read "a'".

Page 485, line 2, "$\log \frac{5.56}{184.27}$" should read "$\log \frac{5.55}{184.28}$".

Page 488, line 9, two lines following Table III should immediately precede the table.

Page 496, line 3, "phosporus" should read "phosphorus."

SOIL SCIENCE

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ENVIRONMENTAL FACTORS INFLUENCING THE ACTIVITY OF SOIL FUNGI[1]

BY

DAVID AUGUSTUS COLEMAN, *Research Fellow, Rutgers College*

INTRODUCTION

The study of soil biology in its relationship to soil fertility has been the subject of research by numerous investigators. Included in this branch of soil science as stimuli for investigation are the many microscopic inhabitants of the soil, viz., algae, bacteria, fungi and protozoa. Up to within comparatively recent times the soil biologist has concerned himself mainly with the study of soil bacteria, giving only casual attention to the other microflora and fauna of the soil.

The work of Russell and Hutchinson (35, 36) of the Rothamsted Experiment Station has given rise to extensive experimentation dealing with the influence of soil protozoa on soil fertility. In time, therefore, we shall have a very comprehensive knowledge of the possible rôle that these organisms might have on the crop-producing power of the soil.

The other microörganisms in this field have not been the subject of so intimate an investigation. Early researches by Müntz and Coudon (30) and likewise Marchal (27) have shown that fungi have the power to decompose organic matter with the production of ammonia. More recently there appeared a paper by McLean and Wilson corroborating the above and also giving additional data as to the effect of the chemical and physical properties of the soil on the activity of soil fungi. A few investigators have also endeavored to demonstrate the azofying (21) and azotification powers of these organisms. The balance of evidence seems to be on the negative side. The early as well as recent investigations on the ammonifying efficiency of soil fungi indicates that they might play an important rôle in controlling soil fertility. It becomes of interest therefore, to gain some insight into the forces which control the life processes of these organisms.

[1] A thesis presented to the faculty of Rutgers College in partial fulfillment of the requirements for the degree of Master of Science in Research, 1916.

Received for publication March 10, 1916.

The subject chosen for research expresses quite clearly the objective to be obtained. Higher plants are dependent in a large measure upon soil microörganisms for their food. Studies then, of the conditions which affect the activity of these organisms are highly desirable.

Historical Resumé

The history of the general subject of the factors that might influence the activity of soil fungi is not of an extended nature. A survey of the literature seems to indicate that the reaction of the medium has much to do with the activity of these organisms. Marchal (27) relates that soils having a weakly alkaline or neutral reaction have relatively few fungi. Ramann (34) and his co-workers have shown, that in a forest soil with which they experimented the proportion of fungi to bacteria was 3 to 1! These investigators also found high numbers of fungi in soils especially rich in humus and of a high acid reaction. Fischer (8), Faelli (9), Oudmann and Köning (31) and others have also recorded the presence of fungi in rich acid soils.

The relationship of acidity to the numbers and activity of soil fungi is quite favorably brought out in a paper by Hall, et al (12), of the Rothamsted Experiment Station. These authors give data showing the relatively large numbers of fungi in the plots that had become acid on account of continuous application of ammonium sulphate. The presence of organic matter in the soil also seems to be a factor influencing the activity of soil fungi. Marchal (30) gives data recording the presence of relatively few fungi in soils that had been extensively cultivated, or soil containing a small proportion of organic matter. Fischer (8), on the other hand, found that the addition of manure resulted in an excellent medium for the activity of soil fungi. More recently, McLean and Wilson (26) give figures to show the influence of the quality of the organic matter on the activity of soil fungi. The same authors further indicate that the chemical composition of the soil also controls the activity of these organisms. This was especially true when soluble phosphates where present. Numerous other investigators among whom are Löhnis (25), Gerlach and Vogel (11), Jensen (13), Butkewitsch (2), have shown the favorable influence of ammonium salts and of nitrate upon soil fungi.

A recent paper of Waksman and Cook (39), in which the authors endeavor to study the moisture factor in relation to a representative of each of three groups of prominent soil fungi, leads to the conclusion that the optimum moisture content for soil fungi activities is near the optimum for the soil.

It is of interest to note in passing that Kappen (14) and lately Löhnis (25), have shown the possibility of fungi aiding the transformation of cyanamid nitrogen into ammonia. It is evident, then, that much more data needs to be tabulated before any definite conclusions can be drawn regarding the activities of these organisms.

METHODS USED

The soil culture method as recommended by Lipman and Brown (22) for the measurement of the activities of soil microörganisms, modified by the sterilization of the soil was employed throughout this work. Ammonia production and its subsequent accumulation has been shown to be a measure, within certain limits of biological activities. In discussing results in this paper the production of ammonia will be used synonomously with activity, i. e., increased activity meaning increased accumulation of ammonia.

One-hundred-gram portions of soil were weighed out, quantities of organic matter equivalent to 155 mg. of nitrogen were added to each portion and the whole thoroughly shaken in a hand shaker identical with those used for the preparation of egg drinks at soda fountains. This device is fully described by us in another place (18). The contents of the shaker were transfered to a 200-c.c. Erlenmeyer flask and the proper moisture supplied, taking cognizance of the amount of moisture lost in the sterilization process. The flasks were then plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 15 minutes. It has been shown by Coleman, Lint and Kopeloff (3) that this was the only means at present that we have for totally eliminating other biological factors. Upon cooling, the flasks were inoculated with 1 c.c. of a liquid culture of fungus spores.

After incubating 7 days at 22° C. the soil was transfered to copper flasks with 250 c.c. of tap water, a piece of paraffin and an excess of heavy magnesium oxide added, and the ammonia distilled over into N/10 HCl and titered with N/10 NaOH, using cochineal as the indicator.

The fungus spores were obtained by inoculating 100 c.c of Cook and Taubenhaus' (6) medium No. 2 with some of the fungus to be studied and incubating at 22° C. until an abundance of spores had been formed. This usually took from 7 to 12 days, species of *Penicillium* and *Trichoderma* requiring longer periods for sporulation.

The composition of Cook's (6) medium is as follows:

Distilled water	1000 c.c.
Glucose	20.00 gm.
Peptone	10.00 gm.
K_2HPO_4	.25 gm.
$MgSO_4$	.25 gm.

At the end of the incubation period the flask containing the fungus was shaken vigorously for 10 minutes to dislodge as large a number of spores as possible. The liquid was then transfered to a second sterile flask containing sand and again shaken to break up clumps of spores, the number of spores was then counted by the method devised by Kopeloff,

Lint and Coleman (17) for the enumeration of soil protozoa. As the fungus spores were not motile the magnitude of the error would be considerably less than noted in the last mentioned article.

As a check upon the purity of the cultures, as well as on the efficiency of sterilization, a sterile tube of bouillon was inoculated with a small portion of the soil at the end of the incubation period. At the end of 24 hours these tubes were examined for bacterial contamination by plating upon Lipman and Brown's (23) synthetic agar.

The Soils Used

The soils used in these investigations were of two types, one a sandy loam designated by the Bureau of Soils of the United States Department of Agriculture as a Norfolk sandy loam and the other a clay loam, known as a Penn clay loam. The Norfolk sandy loam had a lime requirement according to the Veitch method for soil acidity of 400 pounds of calcium oxide per acre, whereas the Penn clay loam recorded a lime requirement of 1700 pounds per acre. In order to compare the soils on an equal reaction basis sufficient calcium carbonate was added to the Penn clay loam to bring it to the same acidity as the Norfolk sandy loam.

Part I

The Influence of Organic Matter on the Activity of Soil Fungi

Series I

It has generally been accepted that the addition of nitrogenous organic matter to the soil, especially that of vegetable origin containing a large proportion of readily decomposable carbohydrates, favors the growth of soil fungi. This fact often expresses itself by the presence of a large mycelial growth upon the surface of the soil in ammonification experiments. It becomes of interest therefore, to note the influence that different sources of organic matter would have upon the activity of soil fungi as measured by pure culture studies in sterilized soil.

Among the substances available for this purpose are many cereal grains, legume seeds, oil cakes, meals, straws, etc. Accordingly, a series was begun in which were used green rye, mature vetch, soybean meal, cottonseed meal, and for the purpose of comparison, dried blood, a material of animal origin. Cottonseed meal and dried blood constitute the major organic nitrogenous constituent of high grade fertilizers. A comparative study of their decomposition is, therefore, of much value as the rate of decay is not only an index of the availability of the material in question, but is also an index of the activity of the organism at hand.

The vetch and rye had approximately the same percentage of nitrogen, 3.42 per cent and 3.81 per cent, respectively. The percentage was high in the rye as it was cut when young. The cottonseed meal and soy-

bean meal had 6.25 per cent and 6.52 per cent of nitrogen, respectively. The dried blood analyzed 12.54 per cent nitrogen.

The Fungi Studied

The fungi studied in this work were some of the more common species found in the soil. One or more of three groups of soil organisms were used. They were as follows: *Aspergillus niger* (van Tieghem); *Penicillium* sp. 10, *Rhizopus tritica, Zygorhynchus Vuilleminii* (Namyslowski) and *Trichoderma Koningi* (Oud.) (?).

Experiment I. The first fungus whose activity was studied was *Aspergillus niger.* This fungus has been the subject of experimentation by previous investigators, who have tried to measure its activity and importance to soil fertility by its power to fix atmospheric nitrogen. No one to the knowledge of the writer, has endeavored to measure its activity as a possible force in soil fertility by its power to transform organic nitrogenous materials into ammonia.

TABLE I

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
1-2	155 mg. N. as Dried Blood....	2.84	2.68	2.74	....	None
3-4	155 mg. N. as Dried Blood....	3.71	4.34	4.02	1.78	1 c.c. Asp. nig.
5-6	155 mg. N. as Cottonseed Meal	3.54	3.37	3.45	....	None
7-8	155 mg. N. as Cottonseed Meal	10.18	11.14	10.61	7.16	1 c.c. Asp. nig.
9-10	155 mg. N. as Soybean Meal...	6.99	5.40	6.19	....	None
11-12	155 mg. N. as Soybean Meal...	15.36	11.52	13.44	7.23	1 c.c. Asp. nig.
13-14	155 mg. N. as Vetch.....	10.61	10.61	10.61	....	None
15-16	155 mg. N. as Vetch.....	10.66	11.14	10.92	.31	1 c.c. Asp. nig.
17-18	155 mg. N. as Rye.....	10.80	9.78	10.29	....	None
19-20	155 mg. N. as Rye.....	3.94	3.65	3.79	—6.50	1 c.c. Asp. nig.

The arrangement of the experiment, the amount of nitrogen recovered, and the spore count of the inoculum are given in Table I. This same arrangement follows throughout this investigation.

An examination of Table I shows us that this fungus is of relatively low activity with the conditions at hand. The organism had very little power to ammonify dried blood, only 1.78 mg. of nitrogen having accumulated as ammonia at the end of the incubation period. Soybean meal seems to be the most favorable material for the activity of this organism, as the largest accumulation of ammonia was noted with this material as the source of food. The amount transformed, however, was very low, being less than 5 per cent of the total nitrogen supplied. No accumulation of note was recorded where vetch was used as the ammonifiable material. Ammonia no doubt was formed, as a very heavy mycelial growth was noted. The nitrogen cleaved was probably transformed into the tis-

sues of the fungus. This was likewise the case when green rye was used. With this material however, an actual loss of 6 mg. of accumulated ammonia was registered.

For the maximum activity of this fungus, organic matter of vegetable origin having a rather narrow carbon-nitrogen ratio seems to be of the most suitable source. Animal organic matter, even of a very narrow C-N ratio as illustrated with dried blood, is not an acceptable source of food for the best activity of this fungus.

Experiment II. The Aspergillaceae constitute a large group of soil fungi. Many of its members have been frequently isolated. A second experiment was, therefore, started in which another one of this group,—a Penicillium was used. The organism belongs to a group of soil Penicillia designated as "group 10" by Mr. S. A. Waksman (40) to whom the author is indebted for its indentification, as well as for the identification of *Rhizopus tritica.*

TABLE II

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF PENICILLIUM SP. 10

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
21-22	155 mg. N. as Dried Blood....	2.68	2.80	2.74	....	None
23-24	155 mg. N. as Dried Blood....	6.09	6.84	6.46	3.72	1 c.c. Peni. sp. 10
25-26	155 mg. N. as Cottonseed Meal	3.54	3.37	3.45	....	None
27-28	155 mg. N. as Cottonseed Meal	13.63	13.03	13.33	9.88	1 c.c. Peni. sp. 10
29-30	155 mg. N. as Soybean Meal...	3.65	3.45	3.55	....	None
31-32	155 mg. N. as Soybean Meal...	11.30	9.74	10.52	6.47	1 c.c. Peni. sp. 10
33-34	155 mg. N. as Vetch.....	10.61	10.50	10.55	....	None
35-36	155 mg. N. as Vetch.....	12.55	13.25	12.90	2.35	1 c.c. Peni. sp. 10
37-38	155 mg. N. as Rye.....	9.36	9.36	9.36	....	None
39-40	155 mg. N. as Rye.....	13.36	14.08	13.72	4.36	1 c.c. Peni. sp. 10

Upon studying the data in Table II it is easily seen that this fungus is also a rather inactive organism as measured by the ammonia accumulated at the end of 7 days. Although a fair growth of mycelium was noted upon the dried blood cultures, only 3.72 mg. of nitrogen accumulated. Cottonseed meal proved to be the most readily ammonified. The green rye cultures had a very large mycelial growth. This may aid in explaining the low amount of ammonia accumulating from the degradation of this material. Vetch was a very poor source of organic matter for this fungus to act upon. Not only did this prove true as measured by ammonia acmulation; but also a survey of the culture flasks showed a very scant mycelial growth.

Experiment III. The third organism studied was *Rhizopus tritica.* At first it was thought that this organism was *Rhizopus nigricans.* When

tested out, however, for the formation of a sexual stage as outlined by Blakeslee it did not form this characteristic. Further analysis showed it to be in all probability *Rhizopus tritica.*

Examining the yields of ammonia, in Table III, accumulated from the decomposition of the several organic nitrogenous materials it can be easily seen that *Rhizopus tritica* is a very active organism in the presence of all the organic materials. Soybean meal was the substance most readily decomposed by this fungus, 39.25 mg. or 25.30 per cent of the total nitrogen supplied, manifesting itself as ammonia at the end of the incubation period. Cottonseed meal was also a very suitable material for an enhanced activity of this organism. On considering the other nitrogenous materials it is patent that green rye and vetch were also quite easily decomposed by the fungus, 22.85 mg. and 16.51 mg. of nitrogen, respectively, having been transformed in 7 days.

TABLE III

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
31-32	155 mg. N. as Dried Blood....	2.68	2.80	2.74	....	None
33-34	155 mg. N. as Dried Blood....	34.13	34.01	34.07	31.33	1 c.c. Rhiz. tritica
35-36	155 mg. N. as Cottonseed Meal	3.54	3.37	3.45	....	None
37-38	155 mg. N. as Cottonseed Meal	37.79	37.24	37.51	34.06	1 c.c. Rhiz. tritica
39-40	155 mg. N. as Soybean Meal..	3.65	3.45	3.55	....	None
41-42	155 mg. N. as Soybean Meal...	41.91	43.69	42.80	39.25	1 c.c. Rhiz. tritica
43-44	155 mg. N. as Vetch.....	11.24	9.78	10.51	....	None
45-46	155 mg. N. as Vetch.....	32.36	32.36	32.36	22.85	1 c.c. Rhiz. tritica
47-48	155 mg. N. as Rye.....	10.80	9.78	10.29	....	None
49-50	155 mg. N. as Rye.....	27.18	26.43	26.80	16.51	1 c.c. Rhiz. tritica

Quite in contrast to the two previously studied forms this fungus was very active with dried blood as a source of energy. The same general preference for organic matter of vegetable origin is at hand, however.

Mycelial growth was especially marked in all the culture flasks. This was noticeably true where cottonseed meal and green rye were used as the sources of ammonifiable material.

Experiment IV. The data recorded in Table IV give still further evidence as to the effect of organic matter upon the activity of soil fungi. The arrangement is the same as in the preceding work. The fungus studied was *Zygorhynchus Vuilleminii* (Namyslowski). In striking contrast to *Rhizopus tritica* and correlating more closely with the members of the Aspergillaceae studied, low activity was evidenced in the presence of dried blood. As a very active organism in the presence of organic matter of vegetable origin, this fungus compares very favorably with *Rhizopus tritica.* Soybean meal and cottonseed meal seem to regulate a

similar activity when placed in the presence of this fungus, soybean meal inducing a somewhat greater activity. Vetch and rye are also materials suitable for a marked activity of this organism. With the conditions at hand vegetable organic matter was ammonified to the greatest extent. Dried blood was not a good source of energy for the best activity of this fungus.

TABLE IV

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N	Av. Mg. N.	Inc. Mg. N.	
61-62	155 mg. N. as Dried Blood....	2.68	2.80	2.74	....	None
63-64	155 mg. N. as Dried Blood....	8.67	8.97	8.82	6.08	1 c.c. Zyg. Vuil.
65-66	155 mg. N. as Cottonseed Meal	3.54	3.40	3.47	....	None
67-68	155 mg. N. as Cottonseed Meal	27.71	28.73	28.22	24.75	1 c.c. Zyg. Vuil.
69-70	155 mg. N. as Soybean Meal...	3.65	3.50	3.57	....	None
71-72	155 mg. N. as Soybean Meal..	30.96	31.08	31.07	27.45	1 c.c. Zyg. Vuil.
73-74	155 mg. N. as Vetch.....	11.24	9.78	10.51	....	None
75-76	155 mg. N. as Vetch.....	27.27	27.29	27.28	16.77	1 c.c. Zyg. Vuil.
77-78	155 mg. N. as Rye.....	9.36	9.36	9.36	....	None
79-80	155 mg. N. as Rye.....	24.50	23.31	23.90	14.54	1 c.c. Zyg. Vuil.

Experiment V. Another group of soil fungi not as yet considered are the Moniliaceae. McLean and Wilson (26) have shown in their work that these organisms were the most efficient ammonifiers of any of the fungi which they studied. One of the Trichodermae which at the present writing seems to be *Trichoderma Koningi* (Oud.), was used. A casual glance at Table V will at once convince one that this organism is exceedingly active in the presence of all five sources of organic matter. In fact it is the most active organism thus far met with and is also in confirmation of the work of McLean and Wilson (26).

TABLE V

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF TRICHODERMA KONINGI

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
81-82	155 mg. N. as Dried Blood....	2.68	2.80	2.74	....	None
83-84	155 mg. N. as Dried Blood....	40.59	41.57	41.08	39.34	1 c.c. Trich. Kon.
85-86	155 mg. N. as Cottonseed Meal	3.54	3.37	3.45	....	None
87-88	155 mg. N. as Cottonseed Meal	42.63	42.33	42.48	39.03	1 c.c. Trich. Kon.
89-90	155 mg. N. as Soybean Meal...	3.65	3.45	3.55	....	None
91-92	155 mg. N. as Soybean Meal...	46.66	45.70	46.18	42.63	1 c.c. Trich. Kon.
93-94	155 mg. N. as Vetch.....	10.61	10.61	10.61	....	None
95-96	155 mg. N. as Vetch.....	30.23	29.23	29.73	19.12	1 c.c. Trich. Kon.
97-98	155 mg. N. as Rye.....	13.63	13.03	13.33	....	None
99-100	155 mg. N. as Rye.....	14.69	17.74	16.21	2.89	1 c.c. Trich. Kon.

Soybean meal was ammonified to the greatest extent. Not only does this hold true in this particular experiment, but it likewise holds true in comparison with all previous experimentation. Of the 155 mg. of nitrogen supplied, 42.63 mg. accumulated as ammonia in 7 days. Cottonseed meal and dried blood seemed to be identical in regulating the activity of this fungus. With rye, on the other hand, very little ammonia accumulated. The activity of the fungus in the presence of this material seemed to be mainly one of building tissue, i.e. consumption of any cleared am-

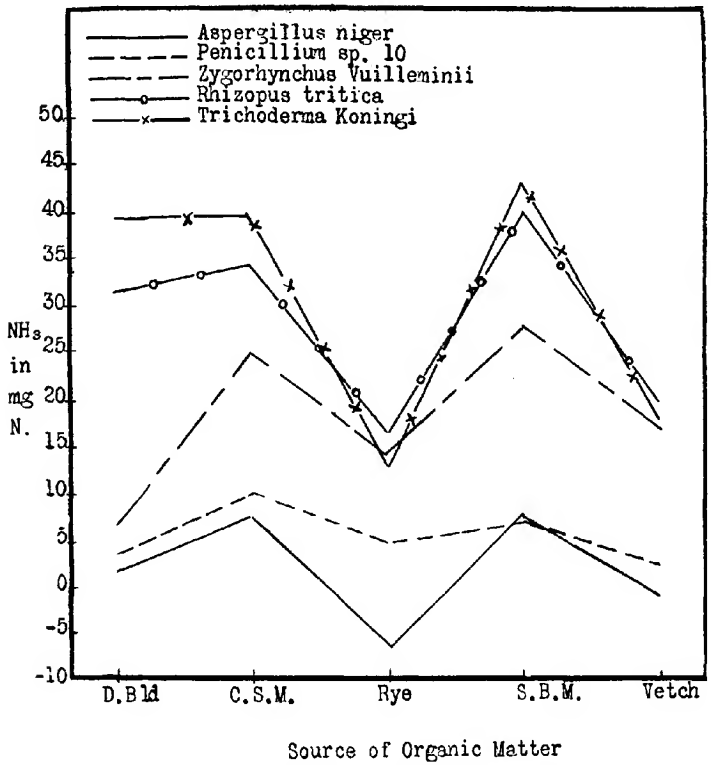

Fig. 1.—Diagram showing the influence of organic matter on the activity of soil fungi.

monia. Vetch was transformed to an appreciable extent, 19.12 mg. of nitrogen accumulating at the end of 7 days. This organism was just as active in the presence of material of vegetable nature as it was in the presence of material of animal nature.

This group of organisms is very closely related to the Penicillia. It is of striking interest to note the wide differences in their activities. The greatest activity, as measured by ammonia accumulation, recorded by the

species of *Penicillium* at hand was 9.88 mg. of nitrogen, whereas the largest accumulation with *Trichoderma Koningi* was 42.63 mg.

The above data would seem to indicate that activity of soil fungi, as measured by their power to decompose organic matter in sterilized soil, would vary with the quality of the material at hand. Materials of vegetable origin were more suitable for the maximum activity of these organisms. This is noticeable where materials of a rather narrow C-N ration are present. Dried blood, however, was quite favorable as a source of energy for the activity of *Rhizopus tritica* and *Trichoderma Koningi*.

A graphic representation of the activities of these organisms in this series is shown in figure 1.

The high activity of the organisms in the presence of the different organic materials is probably due in no small measure to the mechanical condition of the soil. To throw some light upon this phase, as well as to serve as a check upon Series I a second series was started in which a "heavier" clay loam soil was used. Otherwise, the following series is an exact duplication of Series I.

Series II

Experiment VI. That the aeration of the clay loam soil is in all probability inimical to the activity of soil fungi, even though the same source of nitrogen is supplied for the ammonification process, is evident

TABLE VI

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
101-102	155 mg. N. as Dried Blood....	2.77	2.48	2.62	....	None
103-104	155 mg. N. as Dried Blood....	3.21	2.56	2.88	.26	1 c.c. Asp. niger
105-106	155 mg. N. as Cottonseed Meal	4.38	4.00	4.19	....	None
107-108	155 mg. N. as Cottonseed Meal	7.19	6.39	6.79	2.60	1 c.c. Asp. niger
109-110	155 mg. N. as Soybean Meal...	2.63	2.92	2.77	....	None
111-112	155 mg. N. as Soybean Meal...	5.35	....	5.35	2.58	1 c.c. Asp. niger
113-114	155 mg. N. as Vetch.....	9.34	11.51	10.42	....	None
115-116	155 mg. N. as Vetch.....	9.63	9.62	9.62	—.80	1 c.c. Asp. niger
117-118	155 mg. N. as Rye.....	9.20	9.78	9.49	....	None
119-120	155 mg. N. as Rye.....	7.44	7.30	7.37	—2.12	1 c.c. Asp. niger

from a casual glance at the accumulated data. This fact is especially brought out when we consider the activities of *Aspergillus niger* in this series. Table VI records the data. No activity manifested itself as accumulated ammonia when dried blood was used as the source of organic matter. No visible growth of mycelium was noticed on the surface of the soil or within the soil strata. Growth of the organism was noted on all of the cultures which contained the vegetable sources of organic matter. Only two of them, cottonseed meal and soybean meal registered as

having been decomposed to any appreciable extent. It is thus evident that this organism is quite inactive with the conditions at hand in this type of soil.

Although, as was previously mentioned, growth of the organism was noted upon the vetch and rye cultures, no measurable decomposition of these substances took place. In fact, a loss of nitrogen was recorded in the two instances. As was brought out in Series I, vegetable organic matter of high quality is necessary for the best activity of this organism.

Experiment VII. A somewhat identical state of affairs presents itself in the studies with the species of *Penicillium* in this series. Here however, some activity is to be noted. In the presence of dried blood, soybean meal and cottonseed meal again proved to be adaptable sources of organic matter for the greatest activity of this organism. Loss of accu-

TABLE VII

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF PENICILLIUM SP. 10

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
121-122	155 mg. N. as Dried Blood....	2.77	2.48	2.62	....	None
123-124	155 mg. N. as Dried Blood....	4.81	3.65	4.23	1.61	1c.c. Peni. sp. 10
125-126	155 mg. N. as Cottonseed Meal	4.38	4.00	4.19	....	None
127-128	155 mg. N. as Cottonseed Meal	6.07	6.00	6.03	1.84	1 c.c. Peni. sp. 10
129-130	155 mg. N. as Soybean Meal...	2.63	2.92	2.77	....	None
131-132	155 mg. N. as Soybean Meal...	4.81	5.98	5.39	2.62	1 c.c. Peni. sp. 10
133-134	155 mg. N. as Vetch.....	9.24	11.51	10.37	....	None
135-136	155 mg. N. as Vetch.....	6.08	7.73	6.90	—3.47	1 c.c. Peni. sp. 10
137-138	155 mg. N. as Rye.....	9.20	9.78	9.49	....	None
139-140	155 mg. N. as Rye.....	7.84	9.03	8.43	—1.06	1 c.c. Peni. sp. 10

mulated ammonia is again noted where vetch and rye were used as the source of organic matter. This time, however, the greatest loss was recorded where vetch was used. In the previous series the greatest loss was noted where rye was employed.

Experiment VIII. Rhizopus tritica also seems to be affected by the change in the type of soil. Table VIII records the data to show the activity of this organism in the clay loam soil.

Soybean meal was again the best source of organic matter for the maximum activity of this organism. Dried blood was ammonified to a greater extent in this series than was cottonseed meal. In Series I the reverse was true. Vetch was also ammonified to a large extent, 19.22 mg. of nitrogen having accumulated at the end of the incubation period. In comparison with Series I the activity of the fungus in the presence of rye was also somewhat lessened.

TABLE VIII

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
141-142	155 mg. N. as Dried Blood....	6.62	5.43	6.03	....	None
143-144	155 mg. N. as Dried Blood....	21.96	23.14	22.55	16.52	1 c.c. Rhiz. tritica
145-146	155 mg. N. as Cottonseed Meal	6.54	6.24	6.39	....	None
147-148	155 mg. N. as Cottonseed Meal	17.06	19.22	18.14	11.75	1 c.c. Rhiz. tritica
149-150	155 mg. N. as Soybean Meal...	4.51	4.48	4.49	....	None
151-152	155 mg. N. as Soybean Meal...	26.83	25.65	26.24	21.75	1 c.c. Rhiz. tritica
153-154	155 mg. N. as Vetch.....	9.34	11.51	10.42	....	None
155-156	155 mg. N. as Vetch.....	29.60	29.68	29.64	19.22	1 c.c. Rhiz. tritica
157-158	155 mg. N. as Rye.....	9.99	9.63	9.81	....	None
159-160	155 mg. N. as Rye.....	22.07	22.27	22.17	12.36	1 c.c. Rhiz. tritica

Experiment IX. Table IX records the activity of *Zygorhynchus Vuilleminii* in this "heavier" soil type. A glance will point out to the reader that this fungus is not nearly as susceptible to change in soil conditions as were those fungi previously studied. Although the ammonia accumulation is somewhat lessened in comparison with Series I, it is in no case in excess of 6 per cent and in some cases less than 2 per cent. Soybean meal, as in the previous series was best ammonified. Vetch

TABLE IX

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
161-162	155 mg. N. as Dried Blood....	6.62	5.43	6.03	....	None
163-164	155 mg. N. as Dried Blood....	11.44	11.04	11.24	5.20	1 c.c. Zyg. Vuil.
165-166	155 mg. N. as Cottonseed Meal	6.54	6.24	6.39	....	None
167-168	155 mg. N. as Cottonseed Meal	25.30	24.70	25.06	18.61	1 c.c. Zyg. Vuil.
169-170	155 mg. N. as Soybean Meal...	9.90	9.63	9.81	....	None
171-172	155 mg. N. as Soybean Meal...	22.37	22.30	22.33	12.52	1 c.c. Zyg. Vuil.
173-174	155 mg. N. as Vetch.....	6.60	6.83	6.71	....	None
175-176	155 mg. N. as Vetch.....	27.68	28.88	28.23	21.59	1 c.c. Zyg. Vuil.
177-178	155 mg. N. as Rye.....	9.34	11.51	10.42	....	None
179-180	155 mg. N. as Rye.....	25.60	24.60	25.10	14.68	1 c.c. Zyg. Vuil.

was very effectively decomposed, 14.68 mg. of nitrogen having accumulated at the end of 7 days. This is not in excess of the ammonification of cottonseed meal and rye which were more effectively cleaved in the previous series by this organism. Dried blood was again poorly ammonified. It was, however, as well ammonified in this series as in Series I.

Experiment X. Turning our attention to the activities of *Trichoderma Koningi* in this series we find an exceptionally high ammonification of all the organic nitrogenous materials.

A casual glance at the ammonia accumulation shows us that soybean meal was ammonified by this fungus in a perfectly amazing manner. 70.75 mg. or over 45 per cent of the total supplied nitrogen was transformed and accumulated as ammonia in 7 days. This large ammonification is likewise apparent when we observe the figures showing the ammonia accumulation from the decomposition of dried blood and cottonseed meal. Over 50 mg. of nitrogen or 33 per cent of the total supplied nitrogen were transformed into ammonia in 7 days. Twenty per cent of the vetch was likewise transformed into ammonia during the course of the incubation period.

TABLE X

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF TRICHODERMA KONINGI

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
181-182	155 mg. N. as Dried Blood....	6.62	5.43	6.03	....	None
183-184	155 mg. N. as Dried Blood....	57.92	55.31	56.61	50.58	1 c.c. Trich. Kon.
185-186	155 mg. N. as Cottonseed Meal	6.54	6.24	6.39	....	None
187-188	155 mg. N. as Cottonseed Meal	56.22	54.07	55.14	48.75	1 c.c. Trich. Kon.
189-190	155 mg. N. as Soybean Meal...	6.60	6.83	6.70	....	None
191-192	155 mg. N. as Soybean Meal...	74.73	80.40	77.56	70.75	1 c.c. Trich. Kon.
193-194	155 mg. N. as Vetch.....	5.38	5.84	5.61	....	None
195-196	155 mg. N. as Vetch.....	41.15	35.80	38.47	32.88	1 c.c. Trich. Kon.
197-198	155 mg. N. as Rye.....	9.97	9.63	9.80	....	None
199-200	155 mg. N. as Rye.....	25.76	25.65	25.70	15.90	1 c.c. Trich. Kon.

In order to compare the relative activities of these organisms in the presence of the various kinds of organic matter in the different types of soil, the data from Series I and II have been summarized in Table A and also for Series II a graphic representation is given in figure 2. Only the ammonia accumulations after deducting the blank determinations are given. The spore count of the respective inoculations is shown in Table B so that a more careful comparison may be accomplished. It has been shown by Kopeloff (15) in a recent publication that approximately equivalent numbers of spores must be used for inoculation purpose in order to obtain comparable results.

The spore count of the *Aspergillus niger, Penicillium* sp., *Rhizopus tritica* and *Zygorhynchus Vuilleminii* cultures in both series are close enough for a just comparison of the activities of these organisms. Unfortunately the spore counts with *Trichoderma Koningi* are not so close as to warrant so intimate a comparison.

A survey of the data in Table A shows beyond doubt that vegetable organic matter of a fairly narrow C-N ratio is very well adapted to the best activities of the soil fungi studied. Dried blood proved to be an acceptable source of energy for the activity of the organisms in only a few instances, viz., with *Rhizopus tritica* and *Trichoderma Koningi.* This is

true even with a change in environment resulting from the substitution of a second type of soil. Greater activity is to be noted in Series I than in Series II. This is due in a large measure, perhaps, to the greater aeration in Series I.

TABLE A

NITROGEN (MG.) TRANSFORMED FROM ALL SOURCES OF ORGANIC MATTER IN BOTH SOILS

Name of Organism	Sandy Soil					Clay Soil				
	D. Blood	C. S. M.	S. B. M.	Vetch	Rye	D. Blood	C. S. M.	S. B. M.	Vetch	Rye
Aspergillus niger	1.78	7.16	8.23	+.31	-6.50	.26	2.60	2.58	—.80	-2.12
Penicillium, sp. 10	3.72	9.88	6.97	2.35	4.36	1.61	1.84	2.62	-3.47	-1.06
Rhizopus tritica	31.33	34.06	39.25	22.85	16.51	16.52	11.75	21.75	19.22	12.36
Zygorhynchus Vuilleminii ...	6.08	24.75	27.45	16.77	14.54	5.20	18.61	21.59	14.68	12.52
Trichoderma Koningi	39.34	39.03	42.63	19.12	12.89	50.58	48.75	70.75	32.88	15.90

Considering more closely the activity of the various organisms we note that *Trichoderma Koningi* seems to be the most active organism at hand. The greatest activity of this organism was manifested in the presence of soybean meal. This is true in both types of soil. In fact greater activity was noted in Series II than in Series I indicating that the larger inoculation of spores in this series was responsible for the greater decomposition, or perhaps the bettered chemical environment may be an enhancing factor.

TABLE B

NUMBER OF SPORES PER C.C. OF INOCULATING MATERIAL

Name of Organism	Sandy Soil	Clay Soil
Aspergillus niger	209,400	180,000
Penicillium, sp. 10	392,000	400,000
Rhizopus tritica	482,000	464,000
Zygorhynchus Vuilleminii	55,000	56,400
Trichoderma Koningi	192,000	286,000

The activity of this fungus in the presence of dried blood and cottonseed meal is about the same in both types of soil. A very marked activity was also to be noted in the flasks containing vetch and rye as the ammonifiable material.

Rhizopus tritica also enjoys marked activity in soil with dried blood as the source of energy. Its activity is somewhat curtailed, however, in Series II. This organism also exhibits the greatest activity where soybean meal is at hand. Dried blood and cottonseed meal were decomposed to about the same extent. This fungus assumed the greatest activity of all in the presence of vetch and rye in Series I, whereas, *Trichoderma Koningi* attained this rank in Series II.

Zygorhynchus Vuilleminii exhibited very little activity when dried blood was the material to be decomposed. It has been shown by Kopeloff (16) that a very high acidity is necessary for the maximum activity of this organism in the presence of dried blood. This investigator gives data to show a 500 per cent increase in the decomposing power of this organism by raising the acidity of the soil from neutral to an acidity equal

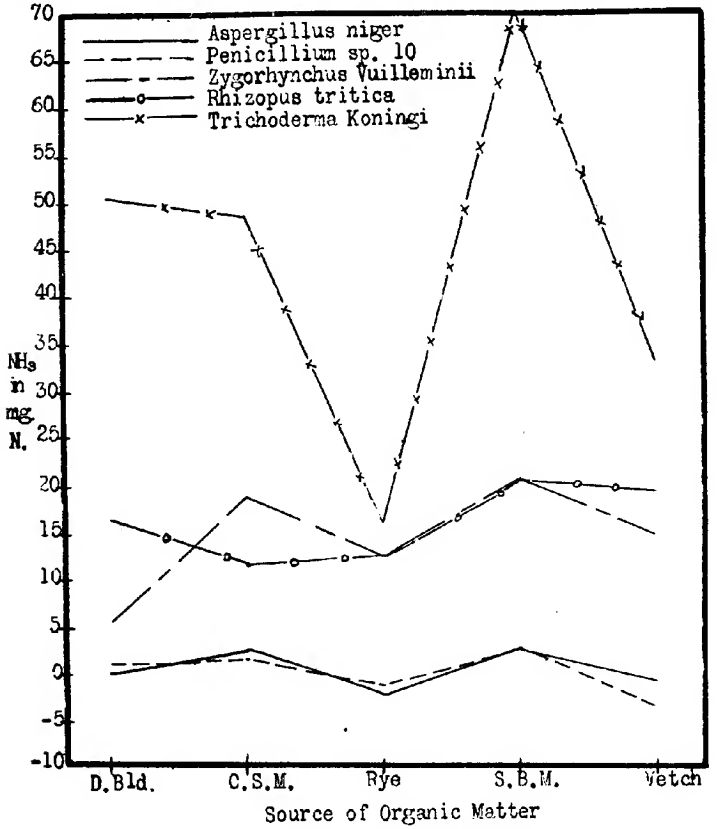

Fig. 2.—Diagram showing the influence of organic matter on the activities of soil fungi.

to 2000 pounds of calcium oxide per acre. This organism was also quite active in the presence of soybean meal and cottonseed meal, a few mg. difference being recorded, however, between the action of the two materials. This fungus was also a very efficient ammonifier of rye and vetch, but little depression in the activities of this organism is to be noted with a change in soil type with these sources of energy.

The two members of the Aspergillaceae were the most drastically affected by the change of soil conditions. Of the two, the species of *Penicillium* seems to be the active organism. Soybean meal and cottonseed meal were both suitable for a measureable activity of these organisms, however. Dried blood was not conducive to the activity of these organisms with the conditions at hand. Kopeloff (16) has, however, shown that *Penicillium* sp. 10 responds very well to a high acid reaction in decomposing this material.

Vetch and rye seemed to promote excessive growth of the organism at the expense of ammonia accumulation.

It is difficult to say that any one organism constantly stands as the most active regardless of soil and ammonifiable material supplied. In other words, it appears, viewing the activity of these organisms from the standpoint of pure cultures, that every organism will do best with a definite combination of soil and organic matter, the quality of the organic matter and the mechanical composition of the soil being important factors. There is, however, one organism which nearly approaches such a description, viz., *Trichoderma Koningi.*

In attempting to explain the various differences in the ammonifiability of the different nitrogenous materials the C-N ration is no doubt an important item.

The reason that soybean meal proved superior to all other materials as a source of food for the activity of the organisms in question is no doubt due to the high percentage of oil and sucrose in the sample. Analyses showed that the amount of oil was approximately 20 per cent, whereas sucrose was present to the amount of 4 per cent.

Cottonseed meal, with approximately the same percentage of nitrogen was ammonified next in order, considering materials of vegetable origin. This material had lost a large portion of its oil and consequently was not available for plant growth. The lower ammonification and subsequent lessened activity is accountable, in a measure, to this fact.

Dried blood, although having a much narrower C-N ratio than any of the other materials was less acceptable, in the majority of cases to the activity of these organisms studied, materials of a much wider C-N ratio being more conducive to enhanced activity.

Considering the two other sources of organic matter, a much wider C-N ratio is present. Of the two, vetch was in general, better suited to fungus activity than rye. The vetch, being a legume, would tend to have more fatty and oily substances than rye. In a measure, therefore, the better activity of the organisms in the presence of this material is accounted for. In all the experiments where these materials were used the organisms exhibited enormous mycelial growths. As this condition must necessarily demand the consumption of food, it is thought that the de-

graded protein was consumed by the fungi in forming the large mycelial growth present.

It is difficult to say just why vetch and sometimes rye should show an actual loss of accumulated ammonia unless the above phenomena take place.

A comparison of the data submitted herein, with that recently published by Lipman (19) of California, working with bacteria indicates that under suitable conditions some fungi are more efficient than bacteria in decomposing nitrogenous organic matter.

Summary of Part I

Results are given which show that:

1. Marked differences were observed in the activity of soil fungi in pure culture in sterilized soil.
2. The soil used was of two types: a sandy loam and a clay loam.
3. Five sources of organic matter were employed in both types of soil, four of vegetable origin and one of animal origin.
4. Organic matter of vegetable origin was in the main more suitable for the highest activity of the soil fungi studied.
5. The quality of the organic matter has much to do with the activity of soil fungi.
6. The most active organism was *Trichoderma Koningi;* the least active *Aspergillus niger.*
7. Marked differences were noticed in the activity of closely related groups of organisms in the presence of the same source of organic matter.
8. Some fungi seem to be more efficient than soil bacteria in decomposing nitrogenous organic matter.
9. Aeration seems to be a controlling factor in the activity of soil fungi.
10. Materials of a rather wide C-N ratio are fairly acceptable for the activity of the organisms noted.

PART II

THE INFLUENCE OF THE MECHANICAL AND CHEMICAL COMPOSITION OF THE SOIL

In order to obtain additional data concerning the activity of these organisms as influenced by the chemical and mechanical composition of the soil the following experiments were made.

One-hundred-gm. portions of the "heavy" clay loam used in the previous experimentation were diluted with pure quartz sand in varying proportions as indicated in the tables to follow. One hundred fifty-five mg. of nitrogen as cottonseed meal were added as ammonifiable material to each portion, the whole thoroughly mixed, the proper moisture added and sterilized in the autoclave as before. The inoculation, incubation and subsequent analyses of the degraded protein was performed as usual.

In order to test the influence of the chemical factors with regard to the activities of soil fungi, varying amounts of the respective chemicals were added to 100-gm. portions of the Penn clay loam. The soil and organic matter were first thoroughly mixed and the salts, when soluble, added in solution to afford better mixing. Where this was not feasible, the chemicals were added in the dry form and thoroughly mixed with the soil.

The chemicals employed were nitrate of soda, chloride of potash, and acid phosphate. The first two were applied in amounts equivalent to 100, 300, and 500 pounds per acre on a 2,700,000-pound basis. The acid phosphate was applied at the rate of 800, 1000 and 1200 pounds per acre. The object being to conform to field conditions as closely as possible.

The fungi used were *Aspergillus niger, Rhizopus tritica, Zygorhynchus Vuilleminii,* and *Trichoderma Koningi.*

Experiment XI. The results obtained with *Aspergillus niger* are given in Table XI.

TABLE XI

THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Soil Mixture	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
201-202	100 gm. loam	9.45	7.20	8.32	....
203-204	90 gm. loam + 10 gm. SiO_2	7.20	9.90	8.55	.23
205-206	80 gm. loam + 20 gm. SiO_2	10.21	12.62	11.41	3.09
207-208	70 gm. loam + 30 gm. SiO_2	15.16	16.13	15.64	7.32
209-210	60 gm. loam + 40 gm. SiO_2	12.98	13.00	12.99	4.67
211-212	50 gm. loam + 50 gm. SiO_2	14.60	16.15	15.38	7.06
213-214	40 gm. loam + 60 gm. SiO_2	20.13	19.11	19.62	11.30
215-216	30 gm. loam + 70 gm. SiO_2	23.66	20.78	22.22	13.90
217-218	20 gm. loam + 80 gm. SiO_2	25.44	26.31	25.87	17.55
219-220	10 gm. loam + 90 gm. SiO_2	36.87	37.31	37.09	28.77

It is apparent from the results here tabulated that this fungus is highly influenced by the increased aeration resulting from the additions of the quartz sand. In the main, increased activity goes hand in hand with increased oxygen pressure. This is quite remarkable when one considers that the increased proportions of sand are in the same degree depressing the beneficial influences of the chemical composition of the soil.

The beneficial effect of increased aeration commences to show itself when 20 per cent of sand has been added to the soil and from here on, with the exception of soil portions 209-210, a gradual increase in accumulated ammonia was recorded. The depression in the above mentioned instance is due, in all probability, to the inefficient mixing of sand and soil.

Experiment XII. The influence which the chemical composition of the soil has upon the activity of this fungus seems to be quite negligible. Table XII records the experimentation. In order to bring out more forcefully the influences of the chemicals applied, the incubation period was lengthened to 12 days. The reader can at once see that any activity manifested, either of a depressed or an enhanced nature, is well within experimental error.

TABLE XII

THE EFFECT OF THE CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Soil Treatment	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
221-222	100 lbs. $NaNO_3$	16.10	15.60	15.85	.....
223-224	300 lbs. $NaNO_3$	16.60	16.50	16.55	+.70
225-226	500 lbs. $NaNO_3$	14.30	16.40	15.35	—.50
227-228	100 lbs. KCl	14.40	16.90	15.65	—.20
229-230	300 lbs. KCl	15.60	16.00	15.80	—.05
231-232	500 lbs. KCl	13.30	14.90	14.20	—1.65
233-234	800 lbs. Acid Phosphate	14.20	.....	14.20	—1.65
235-236	1000 lbs. Acid Phosphate	16.70	14.30	15.50	—.35
237-238	1200 lbs. Acid Phosphate	16.10	15.60	15.85	.....
239-240	No treatment	15.40	16.30	15.85	.....

In view of the fact that this organism was so very responsive to a bettered oxygen supply at the expense of a depressed chemical environment the above results do not appear phenomenal.

Experiment XIII. The activity of *Rhizopus tritica* in the presence of bettered oxygen conditions is recorded in Table XIII. Increased ac-

TABLE XIII

THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Soil Mixture	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
281-282	100 gm. loam	24.78	24.08	24.43	.....
283-284	90 gm. loam + 10 gm. SiO_2	30.10	28.70	29.40	4.97
285-286	80 gm. loam + 20 gm. SiO_2	30.24	31.22	30.73	6.30
287-288	70 gm. loam + 30 gm. SiO_2	30.80	31.92	31.36	6.93
289-290	60 gm. loam + 40 gm. SiO_2	32.62	31.92	32.27	7.84
291-292	50 gm. loam + 50 gm. SiO_2	33.46	34.44	33.95	9.52
293-294	40 gm. loam + 60 gm. SiO_2	36.54	35.14	35.84	11.41
295-296	30 gm. loam + 70 gm. SiO_2	33.60	37.80	35.70	11.27
297-298	20 gm. loam + 80 gm. SiO_2	34.34	37.80	36.07	11.64
299-300	10 gm. loam + 90 gm. SiO_2	32.62	33.76	33.19	8.76

tivity commenced to exhibit itself when the soil was diluted with 10 per cent of sand. As was the case with *Aspergillus niger,* increased activity went hand in hand with increased oxygen supply. While the succeeding

increments of sand did not cause proportionally as enhanced an activity as the initial dilution, nevertheless the increased oxygen pressure was accompanied by a gradual increased activity. It is interesting to note that this activity is constant in spite of the poorer chemical composition of the medium due to the dilution of the soil. That this organism also responds to a better chemical environment is also evident from the data tabulated below.

Experiment XIV. It is interesting to note that all three chemicals in all three applications stimulate activity to about the same degree. Where nitrate equivalent to 100 pounds of nitrate of soda was added a 25 per cent increased activity is to be noted. Higher applications do not cause any depressed or stimulated action.

TABLE XIV

THE EFFECT OF THE CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Soil Treatment	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
301-302	100 lbs. $NaNO_3$	30.66	31.55	31.10	6.67
303-304	300 lbs. $NaNO_3$	31.55	30.66	31.10	6.67
305-306	500 lbs. $NaNO_3$	30.15	30.80	30.47	6.04
307-308	100 lbs. KCl	29.68	31.72	30.45	6.02
309-310	300 lbs. KCl	30.15	30.80	30.47	6.04
311-312	500 lbs. KCl	32.63	30.00	31.31	6.88
313-314	800 lbs. Acid Phosphate	31.92	32.62	32.27	7.84
315-316	1000 lbs. Acid Phosphate	30.38	31.22	30.80	6.37
317-318	1200 lbs. Acid Phosphate	31.26	lost	31.26	6.83
319-320	No treatment	24.78	24.08	24.43	.....

This was likewise the case where potash was applied. The increased activity was about the same as noted with the nitrate salt.

Acid phosphate gave the highest stimulation of all three chemicals when applied at the rate of 800 pounds per acre, a 30 per cent increase resulting. Larger quantities had little influence either in enhancing or depressing the activity of this fungus.

A resumé of the experimentation above reported shows us that this organism at hand is quite responsive to an increased oxygen supply even at the expense of a lowered chemical environment. The fungus, however, also responds very well to a bettered chemical environment. Acid phosphate produces the greatest increase in activity, whereas, nitrate of soda and chloride of potash exert a somewhat identical stimulation. A comparison of the chemical and physical factors indicates, as a whole, that the physical factors are more influential than the chemical factors.

Experiment XV. The organisms previously studied have all shown response to a better oxygen supply. The data in Table XV on the other hand seem to indicate that *Zygorhynchus Vuilleminii* is not very responsive to an increased oxygen supply.

It cannot be stated that this organism is not absolutely irresponsive to an increased oxygen supply as a slightly enhanced effect is to be seen in nearly every case. No marked action as measured by the accumulation of ammonia was noted, however. The greatest activity was noted in almost pure sand, i. e., soil portions 339 and 340. In some cases a decrease is to be noted which may be ascribed to several reasons, i. e., inefficient mixing, inoculation, etc.

TABLE XV

THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	Soil Mixture	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
321-322	100 gm. loam	28.00	28.90	28.45	.....
323-324	90 gm. loam + 10 gm. SiO_2	30.30	29.80	30.05	1.70
325-326	80 gm. loam + 20 gm. SiO_2	29.40	28.80	28.60	.15
327-328	70 gm. loam + 30 gm. SiO_2	28.00	31.70	29.85	1.40
329-330	60 gm. loam + 40 gm. SiO_2	28.90	30.90	29.90	1.45
331-332	50 gm. loam + 50 gm. SiO_2	30.10	27.90	29.00	.55
333-334	40 gm. loam + 60 gm. SiO_2	24.60	.....	24.60	—3.85
335-336	30 gm. loam + 70 gm. SiO_2	32.00	29.75	30.87	2.42
337-338	20 gm. loam + 80 gm. SiO_2	25.80	25.40	25.60	—2.85
339-340	10 gm. loam + 90 gm. SiO_2	34.40	29.10	31.75	3.30

Experiment XVI. Although this fungus does not seem to be responsive to an increased oxygen supply its activity in the presence of various fertilizing elements is quite pronounced as can be seen by consulting Table XVI. Increased quantities of nitrates give a gradual stimulating influence. Where nitrates were added equivalent to 100 pounds of nitrate of soda an increased accumulation of 0.7 mg. of nitrogen is to be noted. Three hundred pounds increased the accumulation to 1.95 mg. of nitrogen and at 500 pounds per acre the increased accumulation was 2.60 mg. It is possible that these results cannot be taken on their face value as indirect denitrification, resulting in the liberation of ammonia could have taken place. It has not been shown however, that this organism is a denitrifier and until such data are recorded the above results would seem to be correct.

This organism is also very responsive to potash fertilization. The greatest stimulation was recorded where KCl equivalent to 300 pounds per acre was present. An approximately 20 per cent greater activity was evidenced here than where no potash was applied.

The greatest response to chemicals was exhibited where acid phosphate equivalent to 1200 pounds per acre was present. An accumulation of ammonia equivalent to 7.55 mg. of nitrogen in excess of the no treatment flasks was recorded under this treatment. In general all three appli-

cations of acid phosphate gave way to greater activity than was induced by the addition of either nitrate of soda or chloride of potash.

TABLE XVI
THE EFFECT OF THE CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	Soil Treatment	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
341-342	100 lbs. $NaNO_3$	29.10	29.20	29.15	.70
343-344	300 lbs. $NaNO_3$	31.20	29.60	30.40	1.95
345-346	500 lbs. $NaNO_3$	30.80	31.30	31.05	2.60
347-348	100 lbs. KCl	33.90	33.90	33.90	5.45
349-350	300 lbs. KCl	34.50	34.60	34.55	6.10
351-352	500 lbs. KCl	33.90	33.30	33.60	5.15
353-354	800 lbs. Acid Phosphate	35.80	34.10	34.95	6.50
355-356	1000 lbs. Acid Phosphate	33.60	34.20	33.90	5.45
357-358	1200 lbs. Acid Phosphate	36.70	35.30	36.00	7.55
359-360	No treatment	28.00	28.90	28.45	.....

To sum up the activities of this organism under various chemical and physical conditions it seems indicative that this fungus is not very responsive to a bettered oxygen supply. This is in support of its activity in Part I under various soil types. Its response to bettered chemical environment is marked however. The greatest activity was manifested in the presence of acid phospate, the next highest in the presence of potash and the least in the presence of nitrate of soda. The chemical factor seems to be of greater importance than the physical factor.

Experiment XVII. In order to ascertain the response of *Trichoderma Koningi* to a bettered oxygen supply Experiment XVII was under-

TABLE XVII
THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF TRICHODERMA KONINGI

Lab. No.	Soil Mixture	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
361-362	100 gm. loam	27.58	27.51	27.54	.....
363-364	90 gm. loam + 10 gm. SiO_2	32.69	33.25	32.97	5.43
365-366	80 gm. loam + 20 gm. SiO_2	29.75	30.59	30.17	2.63
367-368	70 gm. loam + 30 gm. SiO_2	35.07	34.34	34.70	7.16
369-370	60 gm. loam + 40 gm. SiO_2	35.63	37.73	36.63	9.09
371-372	50 gm. loam + 50 gm. SiO_2	35.98	32.55	34.26	6.72
373-374	40 gm. loam + 60 gm. SiO_2	36.23	34.30	35.26	7.72
375-376	30 gm. loam + 70 gm. SiO_2	29.05	30.80	29.92	2.38
377-378	20 gm. loam + 80 gm. SiO_2	25.76	26.34	26.05	—1.49
379-380	10 gm. loam + 90 gm. SiO_2	26.24	38.42	27.38	—.16

taken. Table XVII records the data to show the influence of a bettered oxygen supply. A casual glance at this table evidences the fact that this organism is responsive to an enhanced oxygen supply. An increase in ammonia production is to be noted on the addition of only 10 per cent of quartz sand.

More activity was manifested with greater oxygen pressure, however, as will be seen by consulting the table. When the soil had been diluted with 70 per cent of sand a decreased activity is at hand and where 10 per cent and 20 per cent of soil are present a decreased activity in comparison with the non-diluted soil is noted.

This decrease in activity is due in no small measure to a poorer chemical environment as is evidenced by the effect of the various chemicals upon the activity of this biological factor.

Experiment XVIII. Table XVIII records the results of the influence of various chemicals upon the activity of this fungus. Upon the addition of $NaNO_3$ equivalent to 100 pounds per acre, an enhanced activity was at once recorded. An increase of 7.34 mg. is to be noted here. Larger quantities of nitrate seemed to depress the ammonifying activity of this fungus below that exhibited with the smallest amount.

TABLE XVIII

THE EFFECT OF THE CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF TRICHODERMA KONINGI

Lab. No.	Soil Treatment	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
381-382	100 lbs. $NaNO_3$	35.91	33.81	34.86	7.32
383-384	300 lbs. $NaNO_3$	33.39	29.26	31.32	3.78
385-386	500 lbs. $NaNO_3$	30.87	32.90	31.88	4.34
387-388	100 lbs. KCl	33.10	33.18	33.14	5.60
389-390	300 lbs. KCl	29.96	24.50	32.23	4.69
391-392	500 lbs. KCl	33.60	33.52	33.56	6.02
393-394	800 lbs. Acid Phosphate	30.10	27.30	28.70	1.16
395-396	1000 lbs. Acid Phosphate	29.93	29.12	29.52	1.98
397-398	1200 lbs. Acid Phosphate	27.38	30.03	28.70	1.16
399-400	No treatment	27.58	27.51	27.54	.....

Potash also stimulated activity. The increased ammonia accumulation is not so large as produced by nitrate of soda equivalent to 100 pounds per acre. No increase or depression is to be noted over the initial stimulation.

Acid phosphate in the quantities applied had very little influence upon the activity of this organism. The increased ammonia accumulation, taking into consideration all three applications, is not greater than 2 mg. of nitrogen. The data at hand, then, would seem to indicate a bettered mechanical composition of the soil as well as the presence of soluble nitrates and available potash salts are desirable for the maximum activity of this fungus.

The data in Part II have been summarized in Tables C and D, the results are tabulated as the increase or decrease of nitrogen (in milligrams) accumulated as ammonia over the portions receiving no treatment.

This summary shows us that soil fungi may vary considerably in their oxygen requirements. Some organisms may be extremely responsive to a high oxygen pressure, whereas others may not be at all responsive to increased quantities of this element. Fungi may also have requirements that are gradient between high and low oxygen pressures.

Aspergillus niger exhibited the greatest activity when subjected to increased aeration. This fungus may be representative of those organisms which require an abundant supply of oxygen for their maximum activity.

Rhizopus tritica is representative of those organisms which show an oxygen requirement below that required for the maximum activity of the first discussed fungus.

TABLE C

SUMMARY OF THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF SOIL FUNGI

Organism	Soil alone	10% SiO_2	20% SiO_2	30% SiO_2	40% SiO_2	50% SiO_2	60% SiO_2	70% SiO_2	80% SiO_2	90% SiO_2
Aspergillus niger	.....	.23	3.09	7.32	4.67	7.06	11.30	13.90	17.55	28.77
Rhizopus tritica	.....	4.97	6.30	6.93	7.84	9.52	11.41	11.27	11.64	8.76
Zygorhynchus Vuilleminii ...	.....	1.70	—.15	1.40	0.95	.55	—3.85	2.42	—2.85	3.30
Trichoderma Koningi	.....	5.43	2.63	7.16	9.09	6.72	7.72	2.38	—1.49	—.16

Trichoderma Koningi represents a group that might get along with still less aerated conditions.

In *Zygorhynchus Vuilleminii* we have an approach to those organisms which would be very active in a very narrow atmosphere of oxygen.

The influences exerted by the various fertilizing elements have been summarized in Table D. The increase of nitrogen (in milligrams) over no treatment are reported.

The data indicate that some fungi may be quite unresponsive to chemical fertilization, whereas others may respond very well to these influences. Again the same fertilizing element may influence various fungi quite differently. For example, acid phosphate may enhance the activity of one organism (*Rhizopus tritica*), and decrease the activity of another (*Trichoderma Koningi*). In some cases no influence at all was to be noted from the amounts applied.

As was previously noted *Aspergillus niger* gave no response to any of the chemicals applied. *Zygorhynchus Vuilleminii* manifested considerable increased activity in the presence of all the fertilizing elements. *Trichoderma Koningi* responded well to small applications of nitrate of soda, but this activity was depressed by further additions. Acid phosphate caused very little increase in the activity of this fungus as measured by the accumulation of ammonia.

The greatest response to nitrate fertilization was manifested by *Trichoderma Koningi* when amounts equivalent to 100 pounds per acre of $NaNO_3$ were present. The greatest activity due to potash fertilization

was shown by *Rhizopus tritica*. This organism also evidenced the greatest activity when acid phosphate equivalent to 800 pounds per acre were added to the soil.

A survey of both tables indicates, when one considers a specific organism, that a bettered mechanical composition may be more important in bringing about increased activity than a superior chemical environment, and *vice versa*.

TABLE D

SUMMARY OF THE EFFECT OF CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF VARIOUS SOIL FUNGI

Organism	100 lbs. $NaNO_3$	300 lbs. $NaNO_3$	500 lbs. $NaNO_3$	100 lbs. KCl	300 lbs. KCl	500 lbs. KCl	800 lbs. A. P.	1000 lbs. A. P.	1200 lbs. A. P.
Aspergillus niger	+.70	—.50	—.20	—.05	-1.65	-1.65	—.35	.....	.....
Rhizopus tritica	6.67	6.67	6.04	6.52	6.04	6.88	7.84	6.37	6.83
Zygorhynchus Vuilleminii	.70	1.95	2.60	5.45	6.10	5.15	6.50	5.45	7.55
Trichoderma Koningi	7.35	3.78	4.34	5.60	4.69	6.02	0.98	1.98	1.16

In other instances, both an increased oxygen supply and an enhanced chemical condition are supplementary to greater activity. A more continued study of the chemical and physical factors in controlling fungus activity would seem very desirable in order to obtain more definite information in regard to the influences that these factors may regulate.

Summary of Part II

The more important points in Part II are summarized below.

Under the conditions at hand:

1. Fungi respond to an increased oxygen pressure.
2. The greatest response to oxygen supply was by *Aspergillus niger* and the least by *Zygorhynchus Vuilleminii*.
3. All the fungi studied evidenced different oxygen requirements.
4. A bettered chemical condition of the soil was followed by increased activity, with but one exception.
5. Fungi do not respond alike to the presence of fertilizing materials. The chemical favorable to one may be harmful to another.
6. To some fungi a bettered oxygen supply is of more importance than a bettered chemical supply, and vice versa.
7. Other fungi respond to both a bettered chemical and a bettered physical environment.

PART III

THE INFLUENCE OF MOISTURE UPON THE ACTIVITY OF SOIL FUNGI

Since attention to the well being of beneficial soil organisms is admittedly a vital factor in the maintainance of soil fertility, it becomes as important a question to determine the relationship of a soil moisture con-

tent to its microbiological activities, as to ascertain the relation thereof to the growth of the plant itself. Relatively little work has been done along these lines.

Engberding (27) found that the number of bacteria increased with the water content of the soil until the latter had reached 80 per cent of saturation and that the numbers decreased when moisture was applied in larger amounts.

Lipman and Brown (24) working with a neglected clay loam soil found that ammonification increased with an increase in the moisture content even up to an amount equal to 35 per cent of the dry weight of the soil. They also found that nitrification in the same soil was most active at 15 per cent of moisture and quite perceptible at 5 per cent. Coleman (4) corroborates their results at 15 per cent, but found a marked reduction even at 10 per cent.

Lipman and Sharp (20) in a recent publication record data to show the effect of moisture on the azofication powers of a sandy loam soil. They found this biological process taking place in the presence of 4 per cent moisture and also in what they termed a virtually water-logged soil.

Nothing of any cogency has thus far come to hand which deals with the aspects of the same question with regard to the activity of soil fungi. The following data are the result of an endeavor to throw some light upon this phase of the soil biology problem. The same soil types and soil fungi as previously described in Part I were employed. In this investigation the organic matter was limited to the use of dried blood and cottonseed meal. Thus organic matter from both animal and vegetable sources was employed.

Methods

One-hundred-gm. portions of soil were weighed out, the desired organic matter added and the whole thoroughly shaken, as previously noted, and transferred to 200-c.c Erlenmeyer flasks. Tap water was added in successive portions as indicated in the tables. The flasks were sterilized as before. A preliminary experiment indicated the loss of moisture due to sterilization. By deducting this loss from the sum of the hydroscopic, added, and inoculated moisture, the percentage of actual water in the system was arrived at. It will be readily seem that conditions were created for a soil having a wide variance of moisture conditions.

Experiment XIX. Aspergillus niger having shown itself in the previous work (Part I) as an exceptionally inactive organism in the presence of dried blood, the influence of moisture on its activity was carried out with only one source of organic matter, cottonseed meal. Table XIX shows the arrangement of the experiment. Series I constitutes the sandy loam series. The uniform inoculation was, as in the previous experimentation, 1 c.c of a liquid culture of spores. The spore count was 280,000 per c.c.

The figures seem to show that the strain of *Aspergillus niger* that we are using prefers a comparatively dry medium for its activity. The amount of ammonia accumulating decreases gradually as the moisture content increases. The remarkable fact to be noted is the consistent activity of this organism in the soil portions containing high percentages of moisture.

TABLE XIX

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
	155 mg. N.						1 c.c. spores of
400-401	as C. S. M.	7	27.27	26.05	26.66	23.73	Aspergillus niger
402-403	"	11	23.91	24.45	24.18	21.53	"
404-405	"	13	20.07	21.00	20.53	17.86	"
406-407	"	16	19.60	20.10	19.85	17.03	"
408-409	"	18	18.92	19.35	19.13	16.26	"
410-411	"	21	14.98	14.60	14.79	12.07	"
412-413	"	23	11.72	12.03	11.87	9.20	"
414-415	"	25	13.44	14.25	13.84	10.67	"
416-417	"	28	14.50	14.90	14.70	12.03	"
418-419	"	30	16.90	13.60	15.25	11.58	"
420-421	"	33	14.02	14.00	14.01	10.47	"
422-423	"	35	4.38	4.20	4.29	0.77	"

The highest accumulation was noted with the lowest amount of moisture in the system. Beyond optimum moisture conditions, 13 per cent, a decrease in activity as measured by ammonia accumulation is to be noted. Increasing the amount of moisture beyond 21 per cent does not seem to influence the activity of this organism until a virtually saturated condition is at hand. When this point is reached the activity of this fungus at once ceases.

Experiment XX. *Penicillium* sp. 10 was used in this experiment. As was the case with *Aspergillus niger*, it is quite evident that this fungus also has its optimum, where cottonseed meal is the source of organic matter, at the lower moisture contents. With 7 per cent of moisture in the flasks the greatest activity was noted. This activity is decreased when moisture above 13 per cent is present. Further increments do not cause as depressing an effect upon the activity of this organism as was noted with *Aspergillus niger*. In comparison with this last named fungus this organism seems to desire an atmosphere somewhat more moist for its maximum activity.

Experiment XXI. As a check upon the last experiment another was performed in which the same percentage of moisture was used, but a different source of organic matter, namely dried blood. Table XXI records the data.

At the outset the activity of this fungus in the presence of this source of ammonifiable material was very low. The greatest activity seems to manifest itself along toward the saturation point of the soil. Increased

activity is to be noted with increasing moisture conditions until the highest amount of moisture was present. In contrast to the conditions

TABLE XX

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF PENICILLIUM SP. 10 WITH COTTONSEED MEAL AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
	155 mg. N.						1 c.c. spores of
425-426	as C. S. M.	7	13.38	13.29	13.33	10.40	Penicillium sp. 10
427-428	"	11	13.14	12.11	12.62	9.35	"
429-430	"	13	10.21	10.62	10.41	7.64	"
431-432	"	16	8.93	7.73	8.33	5.50	"
433-434	"	18	8.17	8.93	8.50	5.67	"
435-436	"	21	7.83	....	7.83	5.11	"
437-438	"	23	8.61	7.83	8.22	5.55	"
439-440	"	25	6.57	8.93	7.75	5.08	"
441-442	"	28	12.26	9.03	10.64	7.97	"
443-444	"	30	8.61	12.16	10.38	7.61	"
445-446	"	33	13.50	10.50	12.00	6.16	"
447-448	"	35	7.73	7.93	7.83	4.31	"

prevailing where cottonseed meal was used as the source of organic matter, a very weak activity was exhibited with small amounts of moisture in the system.

TABLE XXI

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF PENICILLIUM SP. 10 WITH DRIED BLOOD AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
	155 mg. N.						1 c.c. spores of
449-450	as D. B.	7	7.28	7.32	7.30	2.82	Penicillium sp. 10
451-452	"	11	7.28	7.70	7.49	4.43	"
453-454	"	13	10.22	10.78	10.50	6.10	"
455-456	"	16	10.64	11.62	11.13	6.53	"
457-458	"	18	11.62	10.92	12.27	6.64	"
459-460	"	21	11.70	10.22	10.71	6.44	"
461-462	"	23	11.34	11.62	11.48	6.83	"
463-464	"	25	11.70	10.22	11.21	6.83	"
465-466	"	28	11.62	11.34	11.49	6.88	"
467-468	"	30	11.34	12.88	12.11	6.25	"
469-470	"	33	12.51	.....	12.51	6.11	"

Experiment XXII. Turning our attention to another group of soil organisms, the Mucoraceae, we find a different phenomenon presenting itself. Table XXII gives us some idea of the activity of *Rhizopus tritica* under varying moisture conditions. The ammonia accumulation at 0.5 optimum, 7 per cent, is nearly as large as that recorded at the highest accumulation. Increasing amounts of moisture, after the soil's optimum is reached had a very slightly depressing effect. When a nearly saturated condition is reached a marked depression is noted. On saturating the soil

very little activity was recorded. The greatest accumulation was at optimum moisture conditions. Again, to be noted in passing is the high activity of these organisms in the presence of exceedingly high moisture conditions.

TABLE XXII

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF RHIZOPUS TRITICA WITH COTTONSEED MEAL AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
	155 mg. N.						1 c.c. spores of
471-472	as C. S. M.	7	34.43	34.59	34.51	31.58	Rhizopus tritica
473-474	"	11	35.35	35.40	35.37	32.70	"
475-476	"	13	36.27	35.15	35.71	33.04	"
477-478	"	16	34.87	33.33	34.10	31.27	"
479-480	"	18	32.67	36.13	34.36	32.50	"
481-482	"	21	36.13	36.41	36.27	33.54	"
483-484	"	23	36.77	36.87	36.82	33.15	"
485-486	"	25	33.04	32.58	32.81	30.23	"
487-488	"	28	32.58	32.85	32.71	30.04	"
489-490	"	30	33.62	32.52	33.07	30.40	"
491-492	"	33	24.85	28.31	26.58	22.74	"
493-494	"	35	9.09	9.65	9.37	5.85	"

Experiment XXIII. To gain further light upon the influence of moisture on the activity of this fungus the above experiment was repeated, dried blood being substituted for cottonseed meal.

TABLE XXIII

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
	155 mg. N.						1 c.c. spores of
495-496	as D. B.	7	18.84	17.59	18.21	13.76	Rhizopus tritica
497-498	"	11	31.07	29.97	30.52	27.45	"
499-500	"	13	31.45	31.45	31.45	27.05	"
501-502	"	16	29.25	32.33	30.79	26.19	"
503-504	"	18	30.93	32.47	31.70	27.77	"
505-506	"	21	31.07	31.07	31.07	26.80	"
507-508	"	23	30.93	29.10	30.03	25.45	"
509-510	"	25	28.88	30.93	29.90	25.60	"
511-512	"	28	30.93	30.65	30.79	26.18	"
513-514	"	30	4.97	5.22	5.08	2.22	"
515-516	"	33	4.38	4.58	4.46	1.06	"

Very little activity is to be noted in the presence of dried blood with low amounts of moisture in the soil contrasting with the results where cottonseed meal was used. Increasing the percentage of moisture however, results in increased activity. When optimum moisture conditions prevail the highest activity is to be recorded. When additional moisture is present a depression in the activity of this organism commences to

manifest itself. High activity is at hand, however, with large percentages of moisture in the system. When a saturated condition is reached the activity of the fungus is exceedingly weak, and when a water-logged condition is reached, very little, if any, activity is to be noted.

In this experiment the greatest activity is again at the soil's optimum moisture conditions. This experiment checks the preceding one very well in this respect. The only striking difference to be noted is that dried blood is ammonified to a less extent by this fungus than cottonseed meal in soil having moisture contents near one-half optimum. This lower activity, in the presence of dried blood with low moisture contents, of both *Rhizopus tritica* and *Penicillium* sp. seems to suggest that more favorable moisture conditions are necessary for the activity of these organisms where dried blood is used as a source of ammonifiable material. On the other hand, cottonseed meal seems to promote high activity even in soil with an exceedingly low moisture content.

Experiment XXIV. Giving our attention to one of the other members of this group, *Zygorhynchus Vuilleminii* we note a very similar arrangement of results. Table XXIV records the data showing the influ-

TABLE XXIV

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII WITH COTTONSEED MEAL AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
517-518	155 mg. N. as C. S. M.	7	29.40	28.35	28.87	25.94	1 c.c. spores of Zygorhynchus Vuilleminii[1]
519-520	"	11	32.40	31.80	32.10	29.43	"
521-522	"	13	31.63	32.08	31.85	28.18	"
523-524	"	16	32.91	32.19	32.56	29.73	"
525-526	"	18	33.40	32.17	32.78	29.91	"
527-528	"	21	31.40	31.40	31.40	28.68	"
529-530	"	23	29.54	32.17	30.85	28.18	"
531-532	"	25	32.45	30.17	31.31	28.64	"
533-534	"	28	32.17	31.72	31.94	29.27	"
535-536	"	30	31.87	31.32	31.59	28.92	"
537-538	"	33	29.79	29.79	29.79	25.95	"
539-540	"	35	9.41	9.25	9.33	5.81	"

[1] 58,000 spores per c.c.

ence of moisture upon the activity of this fungus, when cottonseed meal is used as a source of organic matter. The results seem to be in accord with those secured in the experimentation with *Rhizopus tritica.* With 7 per cent of moisture in the soil we have exactly the same accumulation of ammonia as with 33 per cent or a nearly saturated condition, truly a remarkable adaptation to moisture conditions. On increasing the amount of moisture from one-half optimum to optimum, we have a gradual increase in the activity of this fungus. This activity remains practically

constant with increasing moisture contents until an approximately saturated condition is reached. There is then, upon increased moisture conditions a rapid and pronounced depression in the ammonifying power of this fungus. There seems to be no exact point giving the highest ammonia accumulation. Any point between 11 per cent and 30 per cent seems to be just as favorable as the best moisture conditions for the ammonifying efficiency of this organism.

Experiment XXV. A duplication of the above experiment with dried blood in place of cottonseed meal gives similar results. Table XXV records the data. It is again seen that this fungus is very tolerant of high moisture conditions. At one-half optimum, 2.55 mg. of nitrogen accumu-

TABLE XXV

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII WITH DRIED BLOOD AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Mg. N. Inc.	Inoculation
			Mg. N.	Mg. N.	Mg. N. Av.		
541-542	155 mg. N. as D. B.	7	6.95	7.05	7.00	2.55	1 c.c. spores of Zygorhynchus Vuilleminii[1]
543-544	"	11	8.64	8.50	8.57	5.50	"
545-546	"	13	10.00	10.00	10.00	5.60	"
547-548	"	16	8.79	8.65	8.72	4.12	"
549-550	"	18	7.87	7.87	7.87	3.24	"
551-552	"	21	8.64	8.50	8.57	4.30	"
553-554	"	23	8.90	8.52	8.71	4.13	"
555-556	"	25	9.10	8.64	8.87	4.47	"
557-558	"	28	9.72	9.30	9.51	4.90	"
559-560	"	30	4.79	4.79	4.79	.93	"
561-562	"	33	4.49	4.79	4.49	1.09	"

[1] 58,000 spores per c.c.

lated, and at optimum 5.60 mg. Further additions cause a slight decrease in the ammonifying efficiency of this organism. The accumulation at optimum was nearly as great as it was with 28 per cent of moisture in the soil. The same conditions which concerned the activity of the previous fungi in the presence of dried blood in soils of small moisture contents are at hand here.

Experiment XXVI. The following work was carried out with *Trichoderma Koningi.* An examination of Table XXVI shows that this fungus is about as active in soils of extremely low and high water contents. We received here the largest ammonification of cottonseed meal at extremely low moisture contents of any of the fungi studied. We also received at one-half optimum as much as at saturation. In fact, greater mycelial growth was noted with the higher moisture contents. In contrast to the other fungi studied, this organism is very active in a virtually water-logged soil, 34.56 mg. of nitrogen having accumulated out of the possible 155 mg. at this moisture content!

TABLE XXVI

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF TRICHODERMA KONINGI WITH COTTONSEED MEAL AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Mg. N. Inc.	Inoculation
			Mg. N.	Mg. N.	Mg. N. Av.		
	155 mg. N.						1 c.c. spores of
563-564	as C. S. M.	7	35.94	42.81	39.37	36.44	Trichoderma Koningi[1]
565-566	"	11	49.44	49.40	49.92	46.75	"
567-568	"	13	50.44	50.40	50.42	47.75	"
569-570	"	16	49.41	49.56	49.48	46.63	"
571-572	"	18	50.44	50.40	50.42	47.55	"
573-574	"	21	50.44	49.99	50.21	47.49	"
575-576	"	23	47.37	46.10	46.73	44.06	"
577-578	"	25	49.91	49.16	49.53	46.86	"
579-580	"	28	52.81	56.36	51.58	48.91	"
581-582	"	30	42.67	45.97	44.32	41.65	"
583-584	"	33	41.43	41.88	41.65	37.81	"
585-586	"	35	40.44	35.75	38.08	34.56	"

[1] 278,000 spores per c.c.

Experiment XXVII. As was the case in the previous experiments the above experiment was repeated with dried blood as the source of organic matter.

TABLE XXVII

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF TRICHODERMA KONINGI WITH DRIED BLOOD AS THE SOURCE OF NITROGEN

No. Lab.	Nitrogen Source of	Moisture Per cent	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
	155 mg. N.						1 c.c. spores of
587-588	as D. B.	7	29.91	30.22	30.06	27.32	Trichoderma Koningi[1]
589-590	"	11	37.91	37.17	37.54	34.80	"
591-592	"	13	37.30	39.37	38.33	35.59	"
593-594	"	16	41.30	39.76	40.53	37.79	"
595-596	"	18	45.72	44.55	45.13	42.39	"
597-598	"	21	44.41	50.74	47.57	44.83	"
599-600	"	23	49.19	60.79	54.99	52.25	"
601-602	"	25	57.98	61.83	59.90	57.16	"
603-604	"	28	56.04	60.75	58.39	55.65	"
605-606	"	30	47.32	50.06	48.69	45.95	"
607-608	"	33	10.53	6.81	8.67	6.13	"

[1] 278,000 spores per c.c.

Less ammonification took place at lower moisture contents with dried blood as the source of organic matter than where cottonseed meal was employed. The increase in ammonia formation is more pronounced with this material. The highest ammonification took place with 25 per cent moisture in the system. Although ammonia was found at saturated conditions, its accumulation was not as strong as was the case with cotton-

seed meal. The optimum moisture content for this soil, 13 per cent, does not promote the greatest activity of this organism. Conditions more closly approximate to one and one-half optimum give the highest accumulation of ammonia.

Discussion

The data from Series I have been summarized in Tables E and F. An examination of these tables shows one some interesting facts about the activity of soil fungi as influenced by varying moisture conditions.

TABLE E

ACCUMULATION OF NITROGEN (MG.) FROM THE AMMONIFICATION OF COTTONSEED MEAL

Name of Organism	Percentage Moisture											
	7	11	13	16	18	21	23	25	28	30	33	35
Aspergillus niger	23.73	21.53	17.86	17.03	16.26	12.07	9.26	10.67	12.03	11.58	10.47	.77
Penicillium sp. 10	10.40	9.35	7.64	5.50	5.65	5.11	5.55	5.08	7.97	7.61	6.16	4.31
Rhizopus tritica	31.58	32.70	33.04	31.27	32.50	33.54	33.15	30.23	30.04	30.40	22.74	5.85
Zygorhynchus Vuilleminii ...	25.94	29.43	28.18	29.73	29.91	28.68	28.18	28.64	29.27	28.92	25.95	5.81
Trichoderma Koningi	36.41	46.75	47.75	46.63	47.55	47.49	44.06	46.86	48.91	41.65	37.81	34.56

There seems to be no critical point which is favorable to the maximum activity of all five forms studied. The two members of the Aspergillaceae, on the whole, seem to require a somewhat drier medium for their activities than do the other forms studied.

TABLE F

ACCUMULATION OF NITROGEN (MG.) FROM THE AMMONIFICATION OF DRIED BLOOD

Name of Organism	Percentage of Moisture										
	7	11	13	16	18	21	23	25	28	30	33
Aspergillus niger	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium sp. 10	2.82	4.42	6.10	6.53	6.64	6.44	6.83	6.83	1.88	6.25	6.11
Rhizopus tritica	13.76	27.45	27.05	26.19	27.77	26.80	25.45	25.60	26.14	2.22	1.06
Zygorhynchus Vuilleminii	2.55	5.50	5.60	4.12	3.24	4.30	4.13	4.47	4.90	.93	1.03
Trichoderma Koningi...	27.32	34.20	35.59	37.79	42.39	44.83	52.25	57.16	55.65	45.95	6.13

Aspergillus niger required the driest medium for its maximum activity. The species of *Penicillium* enjoyed one somewhat more moist.

Rhizopus tritica seemed to have its greatest activity at optimum moisture conditions in the presence of both sources of organic matter.

Zygorhynchus Vuilleminii requires a least optimum moisture conditions for its greatest activity. Given this condition, increased moisture has but little influence upon its activities until the oxygen supply is entirely cut off.

Trichoderma Koningi has a truly remarkable adaptation to moisture conditions. This organism was very active at one-half optimum and at saturated conditions. Optimum conditions for the activity of this fungus seem to be at hand when moisture conditions are present equal to one and one-half the soil's optimum.

The accumulated data suggest that soil fungi have a wide variance as to moisture requirements. Some fungi may require a dry medium for their maximum activity as is illustrated by the members of the Aspergillaceae studied. Other fungi, however, may have their maximum activity when the soil's moisture content is at optimum (*Rhizopus tritica*). Still other organisms might be more active under high moisture conditions. *Trichoderma Koningi* is illustrative of this point.

The high activity of these organisms in the presence of excessive moisture might also aid in explaining the fertility of many soils which are endowed with a high water content during a greater portion of the year. Necessarily, in these soils, bacterial activities would be profoundly affected. If the fungi, however, are tolerant of these high moisture conditions decomposition processes would still continue. This phenomenon is illustrated in cranberry bogs which are covered with water a great portion of the year. Many of these are high in fetility and do not respond to fertilizer treatment. May not the fungi be instrumental in causing the high fertility of these soils?

Of peculiar interest are the phenomena previously mentioned relative to the moisture requirements for the activity of fungi in the presence of dried blood, i. e., the smaller ammonification of dried blood at lower moisture conditions in comparison with cottonseed meal under similar moisture conditions. Why this obtains the writer is unable to say. It may be due to some enzymatic action that does not take place under dry conditions.

The cessation of activities when saturated conditions were reached was due no doubt to a cutting off of the oxygen supply.

Summary of Part III

Results are given which show:

1 Wide differences exist in the moisture requirements of soil fungi.

2. *Aspergillus niger* is favored by a very dry medium for its maximum activity.

3. *Rhizopus tritica* is most active at optimum moisture conditions.

4. *Trichoderma Koningi* is favored by a moist medium.

5. *Zygorhynchus Vuilleminii* has a wide moisture adaptability.

6. A greater amount of moisture is necessary to ammonify dried blood than cottonseed meal.

7. Five fungi were studied, two sources of organic matter being used and moisture conditions varying from 7 to 35 per cent.

PART IV

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF SOIL FUNGI

Temperature as well as moisture is one of the more important factors influencing the activity of soil organisms. Seasonal variations in temperature are well known facts. It is obvious therefore, because of these different temperature variations microbial life must be profoundly modified. The optimum temperature which allows the most active growth is quite different for various species.

The majority of organisms, in fact all living organisms, have their optimum temperature between 20° and 40° C. The organisms found in the soil are of various natures. In the summer, soil under the radiation of the sun often reaches high temperatures. A greater number of the organisms will therefore, have their optimum nearer 40° C. than 30° C.

The literature on temperature as a factor influencing biological activities is quite extensive. A detailed resumé is not in place here. In passing it is noteworthy to mention that Conn (5) finds higher numbers of bacteria in the winter than in the summer. Möller (29) observed a slight evolution of carbon dioxide from frozen soils and Wollny (41) has data to show that a rise in the soil's temperature is accompanied by an increased production of carbon dioxide. It is quite evident then, that biological activities are not suspended at relatively low temperatures and are enhanced by higher temperatures. Considering specific groups of soil bacteria, one finds that nitrification is feeble at 5° C., perceptible at 12° C., and optimum at 37° C. At 15° C. the breaking down of organic matter is fairly rapid and at 25° C. we find the optimum for many species. Ammonification has been shown by Marchal (27) to be extremely feeble at 5° C. At 20° C. ammonia production was marked and at 30° C. the maximum production was reached.

In a recent publication Traaen (38) has endeavored to study the temperature requirements of some of the soil fungi which he isolated. His method was to measure the radical growth of the organism when grown under various temperatures upon agar plates. The only organism related to those studied here was *Trichoderma Vignorium* which had its optimum temperature at about 18° to 20° C.

No other data having come to hand bearing upon this question it becomes of interest to note just what effect different temperatures would have on the activity of soil fungi. Consequently a series of experiments was outlined to test the activity of the fungi previously studied under various conditions of temperature. The thermal points chosen were 6° to 8° C., 15° to 17° C., 22° to 25° C., 30° C. and 38° C.

Cottonseed meal, having been shown to be an excellent source of organic matter for the activity of soil fungi, was used as the ammonifiable material. The same soils were used as previously described. The fungi

used were those named in Part I. Methods of procedure, analyses, etc., were identical with the previous work.

Series I

Experiment XXVIII. *Aspergillus niger* was the first organism studied. A survey of Table XXVIII will show the reader the wonderful response that this fungus exhibits to high temperature conditions. At 6° to 8° C. no ammonification was detected. Very little

TABLE XXVIII

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF ASPERGILLUS NIGER IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
711-712	6-8	3.30	2.91	3.10	....	1 c.c. spores of Aspergillus niger
713-714	15-17	3.34	2.96	3.15	....	"
715-716	22-25	11.10	9.70	10.40	7.30	"
717-718	30	57.00	58.90	57.95	54.85	"
719-720	38	75.90	78.30	77.10	74.00	"

growth was present in the flask. It was barely noticeable microscopically. At 15° to 17° C., growth was present. The organism was very inactive, however, as no accumulated ammonia could be detected. A slightly enhanced activity was manifested at 22° C. Where the temperature was elevated to 30° C. the fungus became exceedingly active. The amount of ammonia accumulating being nearly 8 times as great as was present at room temperature or 22° C. On raising the temperature to 38° C. still greater activity was to be noted, 74 mg. of nitrogen having accumulated at the end of the incubation period. The optimum temperature for this organism is very high. Perhaps even beyond 38° C. as no diminution of activity was recorded in comparison with 30° C. Marshall (28) is authority for the statement that some strains of this organism enjoy a temperature of 44° C. Also to be noted, is the fact that this organism exhibited no activity, indeed no visible growth at the lowest temperature employed. No decomposition was recorded even at the fairly high temperature of 15° to 17° C. Growth, however, was present. The minimum temperature, therefore, for the activity of this organism must be close to 15° C., and the maximum beyond 38° C. This marked preference for high temperatures explains very well the weak activity of this organism at the lower temperatures.

Experiment XXIX. Table XXIX records the data showing the influence of temperature upon the activity of the species of Penicillium at hand.

At the lowest temperature employed this fungus was very inactive as measured by the ammonia accumulated. Growth, however, was present.

It was very meager. No doubt all the ammonia that might have formed was used by the organism for its life process. The greatest activity was noted at 30° C., 31.78 mg. of ammonia having accumulated at this point. Increase in temperature to 38° C. apparently stopped the ammonifying power of this fungus. The optimum temperature for the activity of this organism appears to be between 30° and 38° C. The activity of the organism is approximately 5 times as great at 30° C. as it is at 22° C.

TABLE XXIX

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF PENICILLIUM SP. 10 IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
721-722	6-8	3.34	2.91	3.12	....	1 c.c. spores of Penicillium sp. 10
723-724	15-17	9.59	8.17	8.88	5.41	"
725-726	22-25	10.59	9.59	10.09	6.62	"
727-728	30	31.78	30.60	31.19	28.07	"
729-730	38	3.66	.....	3.66	.....	"

Experiment XXX. Considering the two members of the Mucoraceae we find in both cases a different status of affairs. Table XXX records the effect of temperature upon the ammonifying efficiency of *Rhizopus tritica.*

TABLE XXX

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF RHIZOPUS TRITICA IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
731-732	6-8	4.13	4.27	4.20	0.85	1 c.c. spores of Rhizopus tritica
733-734	15-17	14.66	14.51	14.58	11.13	"
735-736	22-25	37.95	38.70	38.32	34.87	"
737-738	30	48.54	48.09	48.30	44.85	"
739-740	38	19.69	24.71	22.20	18.75	"

As was noted in the experiment with the preceeding fungus, no ammonification took place at 6° to 8° C. No growth manifested itself at this temperature. At 15° to 17° C. considerable ammonia accumulation is obtained. This accumulation is proportional to the increase in temperature until a point between 30° and 38° C. is reached. At 38° C. the accumulation is greater than at 15° C., but is less than at 22° or 30° C. It is quite evident that this organism's activity is favored by rather high temperatures. The optimum temperature seems to be beyond 30° C. and the maximum is reached beyond 38° C. The minimum temperature seems to be between 8° and 15° C. This fungus is a fairly active organism at comparatively low temperatures. It is of interest to note in passing that

Ames (1) has found that 36° C. is the optimum temperature for the Rhizopus causing the storage rot of sweet potatoes.

Experiment XXXI. The activities of *Zygorhynchus Vuilleminii* are in a measure similar to those of the other members of this group. Table XXXI gives the data showing the influence of temperature upon the activity of this fungus. No ammonia or growth was detected at 6° to 8° C. The activity manifested at 15° to 17° C. was the greatest for all of the

TABLE XXXI

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
741-742	6-8	2.89	3.19	3.04	.41	1 c.c. spores of Zygorhynchus Vuilleminii
743-744	15-17	22.73	21.83	22.28	19.63	"
745-746	22-25	29.30	29.15	29.22	25.77	"
747-748	30	56.84	57.04	56.94	53.84	"
749-750	38	4.56	4.56	4.56	1.11	"

fungi studied, at this temperature 19.63 mg. of ammonia having accumulated at this temperature. The largest accumulation was at 30° C. No activity was recorded at 38° C. This fungus seems to be the most active one at hand at a temperature of 15° to 17° C. The optimum is probably between 30° and 38° C. and the minimum between 8° and 15° C.

Experiment XXXII. Taking up one of the other groups of organisms we note that it also, is favored by high temperature. *Trichoderma Koningi* was the organism studied. At 6° to 8° C. no ammonia accumulated. At 15° C. an accumulation of 9.62 mg. of nitrogen was noted.

TABLE XXXII

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF TRICHODERMA KONINGI IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
751-752	6-8	2.48	4.90	3.69	.22	1 c.c. spores of Trichoderma Koningi
753-754	15-17	2.44	13.75	13.09	9.62	"
755-756	22-25	31.21	39.31	37.83	34.38	"
757-758	30	36.40	29.80	30.50	27.03	"
759-760	38	15.00	.....	15.00	11.53	"

This is second in rank to the activity shown by *Zygorhynchus Vuilleminii* at this temperature. The greatest activity was experienced at 30° C. Upon raising the temperature to 38° C., the ammonia accumulation ceased indicating that the maximum thermal point had been reached. In this soil type the optimum temperature for this fungus' activity is in the vicinity of 30° C. The minimum temperature seems to be between 8° and 15° C.

A resumé of the influence of temperature upon the activity of the organisms in this series is graphically represented in figure 3.

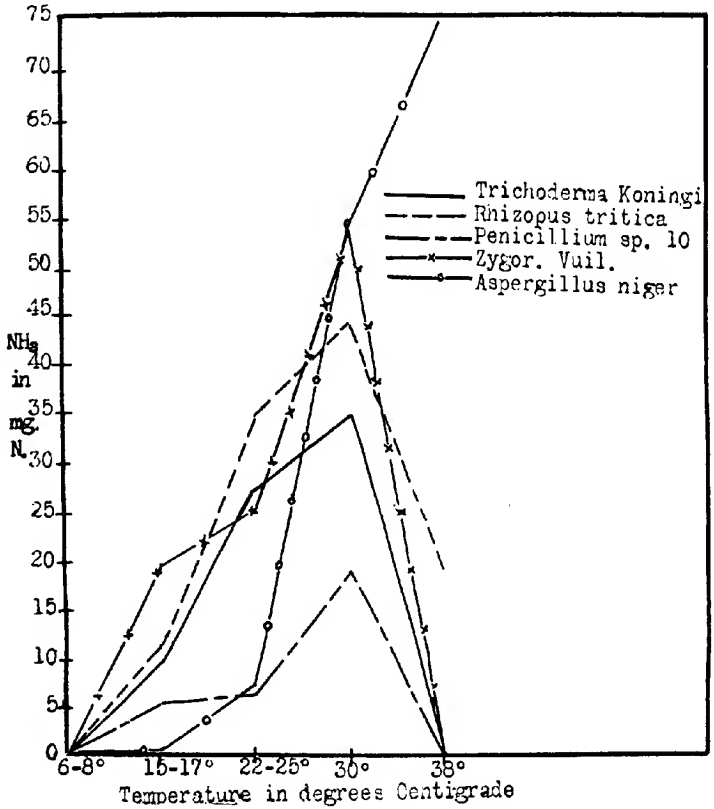

Fig. 3.—Diagram showing the influence of temperature on the activities of soil fungi.

Series II

As a check upon the above series and to give some additional information in regard to the activity of soil fungi under different temperature conditions a second series was executed, in which a heavy clay loam was used in place of the sandy loam previously used. This series is in every respect identical with Series I. Both series received the same inoculation and were incubated at the same time so that all factors are strictly comparable.

Experiment XXXIII. The results reported in Series I, Experiment XXVII are duplicated in every way in this experiment. No activity is

to be detected by ammonia accumulation either at 6° to 8° C. or 15° C., and even at 22° C. very little activity is to be seen. Again, however, on raising the temperature to 30° C., an increased activity is recorded. The response is in about the same proportion as previously stated, perhaps a bit greater. No mycelial growth was present at the lowest temperature in this type of soil. At 15° C. a scant mycelial growth was noted with but few spores present. At 22° C. sporulation had taken place. The 30° C. cultures had a dense black, oily mass of mycelium and spores covering the surface of the soil. When a temperature of 38° C. was reached the greatest activity was again noted, 59.30 mg. of nitrogen being found at the end of 7 days. This is in confirmation of the work in Series 1.

TABLE XXXIII

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF ASPERGILLUS NIGER IN CLAY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
761-762	6-8	3.80	3.60	3.60	....	1 c.c. spores of Aspergillus niger
763-764	15-17	3.80	3.70	3.75	....	"
765-766	22-25	8.80	9.30	9.05	5.50	"
767-768	30	49.90	51.10	50.50	46.90	"
769-770	38	62.27	63.58	62.92	59.30	"

Experiment XXXIV. The data recorded in Table XXXIV give the influence of temperature upon the activity of *Penicillium* sp. 10 in this series. In general the data are in confirmation of Series I. No accumulation of ammonia was noted at 6° to 8° C. A little more than half as much ammonia was accumulated at 15° C. as was accumulated at 22° C.

TABLE XXXIV

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF PENICILLIUM SP. 10 IN CLAY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
771-772	6-8	4.47	6.04	5.25	—.28	1 c.c. spores of Penicillium sp. 10
773-774	15-17	7.46	7.46	7.46	1.83	"
775-776	22-25	7.82	9.88	8.85	3.22	"
777-778	30	23.94	23.16	23.55	18.79	"
779-780	38	4.76	4.76	4.76	—.80	"

The point is again brought out that this fungus has its optimum thermal point at about 30° C. No accumulation of ammonia, or growth of the organism was noted at 38° C. Growth however, was present at 6° to 8° C. although no ammonia accumulated.

Experiment XXXV. The same phenomena hold true with Mucoraceae in this series as was evidenced in Series I. Table XXXV records the data developed from the experimentation with *Rhizopus tritica.* At 6° to 8° C. no growth was noted. A very little ammonia accumulated at

15° to 17° C. Growth of the organism was also very weak. The highest accumulation was obtained at 30° C. An advance in temperature to 38° C. causes a decrease in the activity of this fungus as is evidenced by the ammonia present at the end of the incubation period. A change in

TABLE XXXV

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF RHIZOPUS TRITICA IN CLAY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
781-790	6-8	4.91	4.76	4.83	2.98	1 c.c. spores of Rhizopus tritica
791-792	15-17	7.91	8.21	8.06	2.25	"
793-794	22-25	16.57	18.52	17.54	11.73	"
795-796	30	32.92	31.87	32.39	26.58	"
797-798	38	29.73	29.71	29.72	23.93	"

the soil type causes a change in minimum temperature for this organism's activity. In this series it is nearer 15° C. The optimum temperature is nearer 38° C. Necessarily the maximum would be higher in this series.

Experiment XXXVI. Considering next the activity of *Zygorhynchus Vuilleminii* in this series, we again have evidence to show that this fungus is an efficient ammonifier at fairly low temperatures. Table XXXVI records the data.

TABLE XXXVI

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII IN CLAY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
791-792	6-8	4.44	3.94	4.19	—.73	1 c.c. spores of Zygorhynchus Vuilleminii
793-794	15-17	12.74	12.62	12.68	6.76	"
795-796	22-25	21.01	21.84	21.42	15.50	"
797-798	30	34.79	33.65	34.22	29.30	"
799 800	38	4.46	4.84	4.65	—1.06	"

No ammonia accumulated at 6° to 8° C. At 15° to 17° C., 6.72 mg. was found and at room temperature, 22° C. the next highest activity was exhibited. The highest accumulation of all was found at 30° C. A temperature of 38° C. was inimical to the activity of this fungus. The optimum temperature again seems to be within 30° to 38° C., and the minimum between 8° and 15° C. This experiment is in confirmation of the work in Series I.

Experiment XXXVII. Trichoderma Koningi also exhibits the same order of activity in this series. Table XXXVII tabulates the data. Here again 30° C. is the optimum temperature. No activity was noticed at

38° C. More activity was noted at 15° C. with this organism than was exhibited by any of the other organisms at this temperature.

TABLE XXXVII

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF TRICHODERMA KONINGI IN CLAY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
801-802	6-8	4.46	4.49	4.47	—1.72	1 c.c. spores of Trichoderma Koningi
803-804	15-17	13.21	13.63	13.42	7.02	"
805-806	22-25	36.59	35.40	35.99	33.50	"
807-808	30	52.83	51.90	52.36	49.24	"
809-810	38	4.65	4.53	4.59	—.13	"

In order to bring out the effect of temperature on the activity of the fungi studied the data from both series have been summarized in Table G and the results of Series II are graphically represented in figure 4.

TABLE G

SUMMARY TABLE SHOWING THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF SOIL FUNGI

Name of Organism	Ammonia accumulated (mg. N.) Sandy Soil					Ammonia accumulated (mg. N.) Clay Soil				
	6°-8°	15°-17°	22°-25°	30°	38°	6°-8°	15°-17°	22°-25°	30°	38°
Aspergillus niger	.....	.....	7.30	54.85	74.00	.....	.....	5.50	46.90	59.30
Penicillium sp. 10	.....	5.41	6.62	28.07	.....	—.28	1.83	3.22	18.79	—.80
Rhizopus tritica	.85	11.13	34.87	44.85	18.75	—.98	2.25	11.73	26.58	23.93
Zygorrhynchus Vuilleminii ..	.41	19.63	25.77	53.84	.....	—.73	6.76	15.50	29.30	—1.06
Trichoderma Koningi	.22	9.62	27.03	34.38	11.53?	—1.72	7.02	33.50	49.24	—1.30

Discussion

The data appended in Table G indicate a rather narrow temperature range for the activity of soil fungi. No activity as evidenced by ammonia accumulation, was noted in all ten trials at 6° to 8° C. In some cases a loss is noted which may be attributed to consumption of any accumulated ammonia.

At 15° to 17° C., *Penicillium* sp. 10, *Trichoderma Koningi* and *Zygorhynchus Vuilleminii* all showed marked activity. The optimum temperature for the majority of the fungi studied was around 30° to 35° C. It is the opinion of the writer that this temperature should be chosen for studies with soil fungi as the maximum activity of the forms studied was noted here.

Considering some specific instances, *Rhizopus tritica* was very active at 38° C., whereas all the other organisms with the exception of *Aspergillus niger* were inactive at this degree of heat.

At 30° C. *Aspergillus niger* was the most active organism.

The greatest activity at 15° to 17° C., was shown by *Zygorhynchus Vuilleminii*, the next greatest activity by *Trichoderma Koningi*.

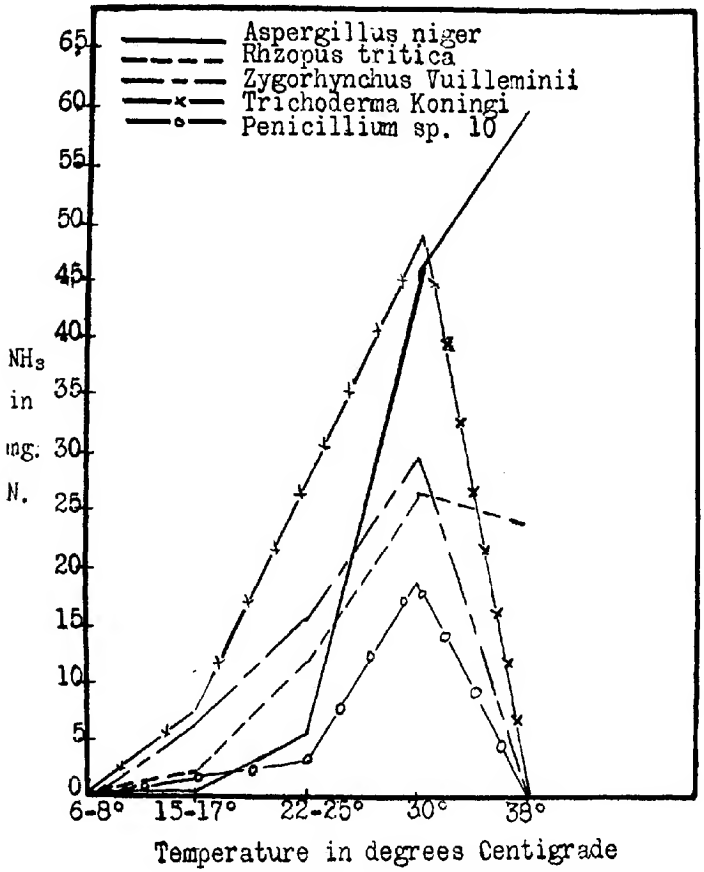

Fig. 4.—Diagram showing the influence of temperature on the activities of soil fungi.

Summary of Part IV

To summarize the work in Part IV the following is tabulated:

1. A 7-day incubation period, one source of organic matter and two types of soil were used as standards.
2. Five species of fungi were experimented upon.

3. Five temperatures were employed; 6° to 8° C., 15° to 17° C., 22° C., 30° C. and 38° C.

4. The data indicate that soil fungi have a narrow temperature range.

5. No activity was recorded in any case at 6° to 8° C. as measured by ammonia accumulation. Growth however was noted in several instances, with a loss of accumulated ammonia.

6. The greatest activity at a temperature of 15° to 17° C. was exhibited by *Zygorhynchus Vuilleminii.*

8. The highest accumulation at a temperature of 22° to 25° C. was shown by *Rhizopus tritica* in both types of soil.

9. At 30° C. *Aspergillus niger* was the most active organism.

10. The minimum temperature for the activity of soil fungi seems to be within 8° and 15° C.

11. The maximum for most of the species at hand was between 30° and 38° C.

12. The optimum temperature for the study of soil fungi should be 30° C. rather than lower temperatures.

13. The soil may be a determining factor in influencing the heat relations of soil fungi.

14. *Aspergillus niger* was the only organism to show no activity at 15° to 17° C.

15. *Aspergillus niger* was also the most active at 38° C.

PART V

THE ASSOCIATIVE ACTION OF SOIL FUNGI AND SOIL BACTERIA

A never failing factor, bound to influence the activity of soil fungi, is their companion organisms in the soil, the bacteria. These two groups of organisms are always together in all types of soil. Indeed it would be a great rarity to obtain a sample of soil with a pure culture of either group of organisms.

Soils are variable in their character of composition. Some soils are very sandy, or open, others are close and heavy. The amounts and proportions of the mineral elements vary to a considerable extent with the various types of soil. In a similar manner the organic matter in the various soil types is of a varied nature, both as to quality and quantity. When one also considers in addition to the above the wide diversity of soil microörganisms with regard to their attitude to the above mentioned conditions, we can at once conceive of many ways in which existing microbial equilibriums, of which soil fungi and soil bacteria are highly representative, could be variously altered. For example, it can be conceived that the addition of dried blood to a soil, in which the predominating flora is of a fungus character, could so favor the growth of bacteria that the group relationship would be very materially changed, and *vice versa,* if cottonseed meal should be used where the flora is highly bacterial in

nature. One can also conceive of alteration in group relations by a change in soil type, a more open soil affording the development of some species to the depression of others. Within the same soil type various seasonal factors may have an important influence upon various soil groups. The amount and distribution of rainfall within a given season, and even within the same soil mass (5) is bound to effect changes in a manner of which we may never have any conception. In a like manner the variations in temperature may also disturb conditions prevailing within a given soil type.

The work carried out in these experiments will be an endeavor to throw some light upon these aforementioned phenomena. No other work having come to hand pertaining to this phase of the soil biology problem it is hoped that the following experimentation will act as a stimulus for further constructive experimentation along this specific line.

Series I

The Associative Action of B. Subtilis and Zygorhynchus Vuilleminii with Moisture as the Limiting Factor.

The organisms chosen for experimentation were *Bacillus subtilis* and *Zygorhynchus Vuilleminii*. Both organisms are excellent representatives of the two groups of soil organisms. The soils used were the Penn clay loam and a second allotment of Norfolk sandy loam, the latter having a lime requirement of 2200 pounds CaO per acre. The soils were made up to an equal reaction basis by the use of $CaCO_3$. Dried blood and cottonseed meal respectively were used as the source of organic matter. The moisture boundaries are shown in the tables. The work was so outlined that the activities of the organisms in the presence of one source of organic matter and in two soil types are comparable. The incubation, inoculation, etc., were performed the same as in Part I.

Experiment XXXVIII. Table XXXVIII-A records the influence of varying amounts of moisture upon the activity of *B. subtilis*. Cottonseed meal was the ammonifiable material. The soil was the Penn clay loam. As will be seen by consulting the table very little activity was noted until 38 per cent of moisture was in the soil. From this point on however, increasing amounts of moisture result in a greater activity of the organism. The greater amount of moisture affording more free water for bacterial activity.

In Table XXXVIII-B one may again observe the activity of *Zygorhynchus Vuilleminii* under the above conditions of moisture. This organism, as previously noted is very active in a low moisture environment. The activity of this organism increases with the greater amounts of water until 43 per cent of moisture is at hand. Further increments had but slight effect either in depressing or accelerating the activity of this organism.

When one observes the influence that moisture has upon the combined activities of these two organisms some very interesting data present themselves.

TABLE XXXVIII—A
THE AMMONIFYING EFFICIENCY OF B. SUBTILIS AS INFLUENCED BY MOISTURE

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1063-1064	18.70	7.98	7.00	7.49	4.87
1065-1066	23.70	8.36	6.65	7.50	4.42
1067-1068	28.50	7.56	7.70	7.63	4.51
1069-1070	33.20	7.26	8.40	7.83	4.71
1071-1072	38.30	10.36	10.50	10.43	7.31
1073-1074	43.10	14.97	.....	14.97	11.85
1075-1076	48.10	15.06	15.23	15.18	12.06
1077-1078	53.80	17.01	19.46	18.23	15.11
1079-1080	58.80	20.23	22.05	21.14	18.02
1081-1082	63.70	23.31	27.07	25.19	22.07
1082-1084	65.70	25.76	26.60	26.18	23.06

Table XXXVIII-C records the data and figure 5 shows graphically the associated and component activities of these organisms.

TABLE XXXVIII—B
THE AMMONIFYING EFFICIENCY OF ZYGORHYNCHUS VUILLEMINII AS INFLUENCED BY MOISTURE

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1085-1086	18.70	23.17	23.87	23.52	20.40
1087-1088	23.70	25.48	26.60	26.04	22.92
1089-1090	28.50	25.34	27.83	26.58	23.46
1091-1092	33.20	27.18	27.65	27.41	24.29
1093-1094	38.30	27.30	25.48	26.39	23.27
1095-1096	43.10	25.83	27.30	26.56	23.44
1097-1098	48.10	25.96	26.23	26.09	22.97
1099-1100	53.80	25.56	26.67	26.11	22.99
1101-1102	58.80	22.94	.....	22.94	19.82
1103-1104	63.70	25.60	24.85	25.23	22.11
1105-1106	65.70	26.74	26.74	26.74	23.62

A consultation of figure 5 shows very clearly that the curve resulting from the combined activities of these organisms is of a decided fungus nature and it seems safe to conclude that the fungus is the more dominating organism with the conditions at hand. It would also seem that the bacterium is more active at the lower moisture conditions when in combination with the fungus than at higher moisture conditions, as the sum of the individual activities corresponds more closely with the sum of the associated activities.

In soil having beyond 43 per cent of moisture, while both organisms are no doubt active, it seems indicative, judging from a consideration of the fungus nature of the curve representing the associated activities,

that that organism was exerting a depressive action upon the activities of the bacterium.

TABLE XXXVIII—C

THE INFLUENCE OF MOISTURE UPON THE ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Inc. over Check	Theoretical Recovery	± Theoretical Recovery
		Mg. N.	Mg. N.	Av.Mg.N.			
1107-1108	18.70	24.57	25.97	25.27	22.15	25.27	3.12
1109-1110	23.70	29.75	26.50	28.12	25.00	27.34	2.34
1111-1112	28.50	27.30	.....	27.30	24.18	27.97	3.79
1113-1114	33.20	31.57	31.22	31.32	28.27	29.00	.73
1115-1116	38.30	32.27	32.27	32.27	29.15	30.58	1.43
1117-1118	43.10	33.11	32.20	32.65	29.53	35.29	5.76
1119-1120	48.10	29.47	32.76	31.11	27.99	35.03	7.04
1121-1122	53.80	31.27	31.36	31.31	28.19	38.01	9.82
1123-1124	58.80	32.13	.....	32.13	29.01	37.82	8.81
1125-1126	63.70	36.12	27.65	31.88	28.76	44.18	15.42
1127-1128	65.70	34.30	35.00	34.65	31.53	46.68	15.15

Experiment XXXIX. In this experiment the influence of moisture upon the activity of *B. subtilis* and *Zygorhynchus Vuilleminii* was de-

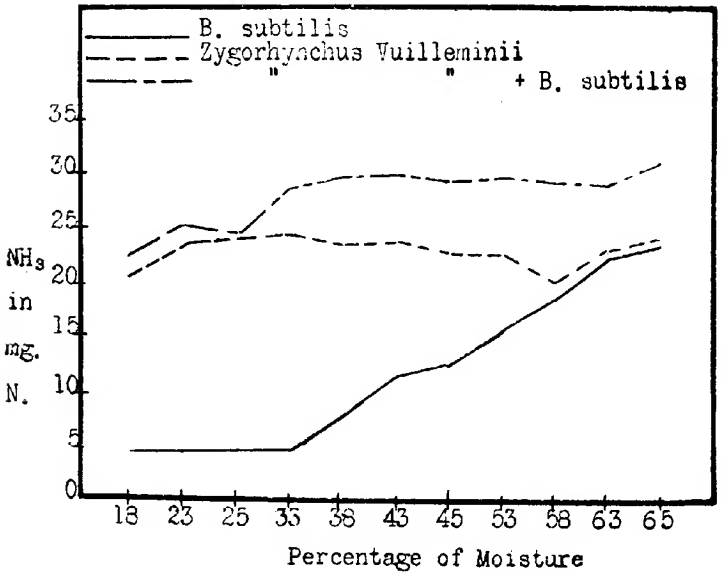

Fig. 5.—Diagram showing the associative action of *B. subtilis* and *Zygorhynchus Vuilleminii.*

termined as in the previous experiment using dried blood in place of cottonseed meal. The type of soil was the same. It is patent from a casual

observation of Table XXXIX-A that the activity of *B. subtilis* in this type of soil was very feeble. This weak activity is not due to a poor inoculation or weak organism as can be seen by consulting Table XL-A to follow, both experiments receiving the same inoculation. Even with

TABLE XXXIX—A

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF B. SUBTILIS

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1129-1130	18.80	5.30	4.70	5.00	1.88
1131-1132	23.70	5.30	5.20	5.25	2.13
1133-1134	28.70	5.20	5.50	5.35	2.23
1135-1136	33.60	6.30	6.30	6.30	3.18
1137-1138	38.60	6.90	6.30	6.60	3.48
1139-1140	43.30	7.60	7.90	7.75	4.63
1141-1142	48.50	8.50	7.50	8.00	4.88
1143-1144	53.40	8.70	8.80	8.75	4.63
1145-1146	58.40	9.25	9.60	9.43	6.31
1147-1148	63.30	7.70	7.70	7.70	4.58

58 per cent of moisture in the system, affording an abundant supply of free water, only 6.31 mg. of nitrogen accumulated at the end of the incubation period.

TABLE XXXIX—B

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1149-1150	18.80	5.40	6.10	5.75	2.63
1151-1152	23.70	11.80	10.60	10.20	7.08
1153-1154	28.70	13.00	...	13.00	9.88
1155-1156	33.60	11.40	14.70	13.05	10.93
1157-1158	38.60	15.30	14.20	14.75	11.68
1159-1160	43.30	16.50	17.30	16.90	13.78
1161-1162	48.50	14.80	16.10	15.45	12.33
1163-1164	53.40	14.90	14.50	14.70	11.58
1165-1166	58.40	10.92	11.63	11.27	8.15
1167-1168	63.30	8.20	7.20	7.70	4.58

The data recording the activity of *Zygorhynchus Vuilleminii* in the presence of dried blood under varying moisture conditions is tabulated in Table XXXIX-B. The maximum point of activity is reached with 43.30 per cent of moisture in the soil. Further increments had very little influence until 63.30 per cent of moisture was at hand.

This fact is in keeping with the previous experimentation. Also to be noted is the higher activity due to the higher acidity of the soil. In this case the acidity was equivalent to a lime requirement of 2200 pounds of CaO per acre.

The data resulting from the combined activites of these organisms are shown in Table XXXIX-C. The data again suggest that in this soil type, with dried blood as the source of energy, that the fungus is again the dominant organism under nearly all the conditions of moisture.

At a moisture content of 18 per cent the sum of the combined activities is approximately equal to the component activities, indicating that at this figure both organisms are equally active.

TABLE XXXIX—C

THE INFLUENCE OF MOISTURE UPON THE ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Inc. over Check	Theoretical Recovery	± Theoretical Recovery
		Mg. N.	Mg. N.	Av.Mg.N.			
1169-1170	18.80	9.50	8.50	9.00	5.88	4.51	1.37
1171-1172	23.70	14.00	12.80	13.40	10.28	9.21	1.07
1173-1174	28.70	15.30	16.50	15.90	12.78	12.11	.67
1175-1176	33.60	18.40	18.50	18.45	15.33	14.01	1.32
1177-1178	38.60	16.00	25.10	20.55	17.43	15.16	2.27
1179-1180	43.30	17.00	17.30	17.15	14.03	18.41	4.38
1181-1182	48.50	16.50	.....	16.50	13.38	17.21	3.83
1183-1184	53.40	16.00	14.60	15.30	12.18	16.21	4.03
1185-1186	58.40	9.40	11.30	10.35	7.23	14.46	7.23
1187-1188	63.30	8.10	10.30	9.20	6.08	9.16	3.08

This phenomenon is also poignant until a point of moisture equal to 43 per cent is present. From here on, however, the activity of the fungus seems to be the predominating one as the curve (fig. 6) at these moisture contents is very "fungoid" in nature, falling off at 58 per cent and 63 per cent, respectively, as was characteristic of the fungus, whereas *B. subtilis* increased at the next to the highest moisture content and was depressed more gradually at 63 per cent.

A resumé of the data expressing the activities of these two organisms in the presence of dried blood and cottonseed meal suggests very strongly the possibility that variations in the moisture supply of the soil, or even, unequal distribution of the moisture already in the soil may influence to a considerable extent various groups of soil microörganisms. In both cases, e. g. in the presence of dried blood and cottonseed meal the activity of the fungus was accelerated by an increase in the moisture supply to a greater extent than was that of the bacterium. The activity of *B. subtilis* was greater in the presence of cottonseed meal than in the presence of dried blood. The same is true with *Zygorhynchus Vuilleminii*. The fungus, as well as the bacterium was more active at lower moisture contents in the presence of cottonseed meal than was the case where dried blood was used as the source of organic matter, which fact confirms the observations previously noted in Part III.

A consideration of the associated activities suggest an advantageous relationship existing in the presence of low amounts of moisture. Large

quantities of water (40 to 50 per cent) seemed to favor the activity of the fungus, although it is very probable that both organisms are active. *The trend of the plotted curves as compared with the component curves,* indicates, in the main the above mentioned phenomena.

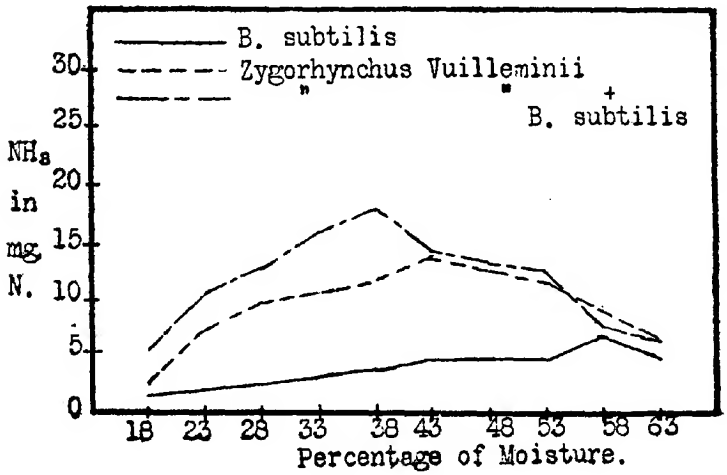

Fig. 6.—Diagram showing the influence of moisture on the associative activities of *B. subtilis* and *Zygorhynchus Vuilleminii.*

Experiment XL. A more open soil with its accompanying poorer chemical environment and increased oxygen supply might *a priori,* influence to a considerable degree, existing group relationships in a manner quite foreign to those occurring in a heavier soil. In order to throw some

TABLE XL—A

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF B. SUBTILIS

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1191-1192	11.80	8.10	8.90	8.50	6.00
1193-1194	15.80	13.20	13.20	13.20	10.70
1195-1196	19.80	15.50	22.60	19.05	16.55
1197-1198	24.20	22.40	20.10	21.25	18.65
1199-1200	29.70	28.70	29.80	29.25	26.75
1201-1202	34.10	10.50	8.80	9.68	7.15
1203-1204	38.60	8.20	8.40	8.30	5.80

light upon this phase of the problem the Norfolk sandy loam as previously described, was used, two sources of organic matter being employed.

Table XL-A records the ammonifying efficiency of B. subtilis in this type of soil with dried blood as the source of organic matter. A glance at Table XL-A evidences the fact that both a change in the type of soil, as well as a greater supply of moisture, was very favorable to the ammonifying efficiency of this organism, an accelerated activity going hand in hand with increased amounts of moisture until a moisture content of 30 per cent or over double optimum conditions are at hand. A moisture content beyond this at once decimates the activity of this bacterium. It should be stated that the source of ammonfiable material was dried blood.

TABLE XL—B

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1205-1206	11.80	7.30	7.80	7.55	5.05
1207-1208	15.80	8.40	7.50	7.95	5.45
1209-1210	19.80	9.60	15.00	12.30	9.80
1211-1212	24.20	11.00	12.70	11.60	9.10
1213-1214	29.70	10.40	10.90	10.65	8.15
1215-1216	34.10	4.40	5.20	4.80	2.30
1217-1218	38.60	4.50	4.50	4.50	2.00

Table XL-B tabulates the action of *Zygorhynchus Vuilleminii* with regard to a change of environment and the presence of varying moisture conditions. An observation of the table yields the information that there is a gradually accelerated activity of this fungus until double optimum conditions are reached. Beyond this point very little activity was noted.

TABLE XL—C

THE INFLUENCE OF MOISTURE UPON THE ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Inc. over Check	Theoretical Recovery	± Theoretical Recovery
		Mg. N.	Mg. N.	Av.Mg.N.			
1219-1220	11.80	13.10	13.20	13.15	10.65	11.05	.40
1221-1222	15.80	15.10	15.90	15.50	13.00	16.15	3.15
1223-1224	19.80	21.30	21.70	21.50	19.00	26.35	7.35
1225-1226	24.20	23.00	24.90	23.95	21.45	27.75	6.30
1227-1228	29.70	29.40	24.60	27.00	24.50	34.90	10.40
1229-1230	34.10	7.80	.....	7.80	5.30	9.45	4.15
1231-1232	38.60	7.00	7.80	7.40	4.90	7.80	2.80

The figures resulting from the combined activities of these organisms in this type of soil and this source of organic matter, dried blood, have been recorded in Table XL-C. As was the rule in the previous experiments curves for the activities of the organisms have been drawn and are presented in figure 7.

The figures for the sum of the component and associated activities are approximately equal at the lowest moisture contents suggesting an advantageous relationship with very little if any antagonism. Conditions of 10 per cent and 15 per cent of moisture are conducive to an antagonistic action. The associated curve (fig. 7) is in the main bacterial in nature evidencing the point that the bacterium is the dominant power in all cases.

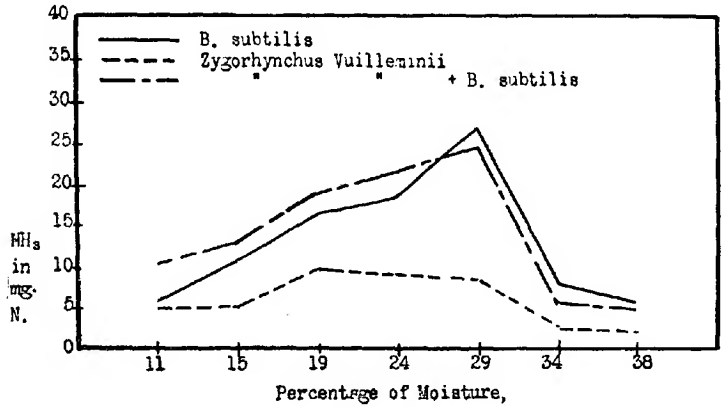

Fig. 7.—Diagram showing the influence of moisture on the associtive activities of *B. subtilis* and *Zygorhynchus Vuilleminii.*

However, as the sum of the associated activities, is generally speaking lower than that recorded by *B. subtilis* acting alone, one is forced to the conclusion that the fungus is depressing the activity of the bacterium. Just how great this depression is can not be accurately judged. A curve plotted to show the difference between the combined and theoretical recovery would also be bacterial in nature. It may be that the magnitude of depression is in this order. A bacterial count, with and without the presence of the fungus would probably clinch the magnitude of antagonism in a very decisive manner.

In order to bring out the influence of a change in organic matter upon the relationships of these two organisms as influenced by moisture conditions in this type of soil experiment XLI was made. Cottonseed meal was used in place of dried blood.

Experiment XLI. Table XLI-A is a record of both change in soil type and a variation of moisture conditions upon the ammonifying efficiency of *B. subtilis.* An observation of the data shows at once the beneficial influences due to a change of environment upon the activity of this fungus. A comparison with Experiment XXXVIII brings this out very

forcefully. Whereas, in Experiment XXXVIII the maximum ammonification was approximately 10 mg. of nitrogen, the maximum in this experiment is nearer 50 mg. It is needless to say that both soils received the same inoculation. The response of the organism to additions of moisture is also more pronounced in this experiment.

TABLE XLI—A

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF B. SUBTILIS

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1233-1234	5.80	6.30	7.00	6.65	3.80
1235-1236	11.70	10.36	11.76	11.06	8.21
1237-1238	15.70	26.11	22.96	24.53	21.68
1239-1240	19.60	33.32	30.80	32.06	29.21
1241-1242	24.70	39.10	40.32	38.71	35.86
1243-1244	29.60	36.68	46.69	41.67	38.82
1245-1246	34.30	46.27	45.78	46.03	43.18
1247-1248	38.30	.....	46.34	46.34	43.49

In Table XLI-B the ammonifying efficiency of *Zygorhynchus Vuilleminii* under these new conditions has been recorded. In direct contradiction to all the previous experimentation, the activity of this organism persists with succeeding increments of water, the highest activity being noted with 40 per cent of moisture in the system. No explanation can be given for the phenomenon.

TABLE XLI—B

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1249-1250	5.80	20.58	25.90	23.24	20.39
1251-1252	11.70	32.41	34.79	33.60	30.75
1253-1254	15.70	34.09	34.09	34.09	31.24
1255-1256	19.60	39.76	37.80	38.78	35.93
1257-1258	24.70	36.75	42.77	39.76	36.91
1259-1260	29.60	45.15	47.88	46.51	43.66
1261-1262	34.30	41.07	50.89	45.98	43.13
1263-1264	38.30	49.35	48.37	48.86	46.01

When the two organisms are combined and their activity measured by ammonia production we find antagonism operating even with 5 per cent of moisture in the soil. It is a clear case of the activity of the fungus being depressed by the bacterium. This phenomenon also holds true with the soil portions containing 10 per cent and 15 per cent of moisture (see Table XLI-C).

Further conclusions are not permissible as the figures for the individual activities are, in general, higher than the results obtained with the

combined activities. It might be remarked, parenthetically, that the microscropical appearance of the fungus was very meager in the flasks where both organisms were acting in association.

TABLE XLI—C

THE INFLUENCE OF MOISTURE UPON THE ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Inc. over Check	Theoretical Recovery	± Theoretical Recovery
		Mg. N.	Mg. N.	Av. Mg. N.			
1265-1266	5.80	14.56	18.13	16.34	13.49	24.19	10.70
1267-1268	11.20	29.05	27.58	28.31	25.46	38.96	13.50
1269-1270	15.70	25.48	23.01	24.24	21.39	52.92	31.53
1271-1272	19.60	35.91	47.95	41.93	39.08	65.14	26.06
1273-1274	24.70	44.38	45.15	44.76	41.91	72.77	30.86
1275-1276	29.60	51.80	51.80	51.80	48.95	82.48	33.53
1277-1278	34.30	52.04	50.96	51.50	48.65	86.31	37.66
1279-1280	38.30	52.50	.....	52.50	49.65	89.50	39.85

As the fungus makes a very poor mycelial growth, however, this observation can not be taken as too prolix. The data from this experiment are shown graphically in figure 8.

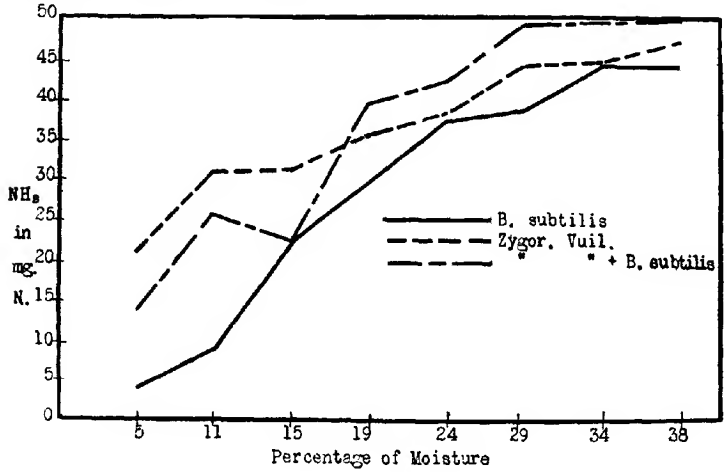

Fig. 8.—Diagram showing the influence of moisture on the associative activities of *B. subtilis* and *Zygorhynchus Vuilleminii*.

A recapitulation of the experimentation carried out in the sandy loam soil with both dried blood and cottonseed meal shows conditions prevailing that are just opposite from those recorded in the clay loam soil. In the experimentation carried out in the clay loam soil, it will be remembered that the fungus was the dominant organism always manifesting the

greatest activity in the presence of both sources of organic matter. The bacterium was the depressing agent. In the experimentation carried out in the sandy loam soil the bacterium was the dominant organism under nearly all conditions having its activity depressed by the fungus.

A general summary of all the experimentation in this series suggests the facts that variations in the moisture contents of the soil may have considerable bearing in altering various biological relationships existing in them. At certain conditions of moisture more antagonistic action was exhibited than at others. In general the antagonistic action of the bacterium upon the fungus was more noticeable at lower concentration of moisture.

Standardizing the moisture factor and the source of organic matter, a change in the type of soil, was also potent in altering relationships between organisms.

The experimentation as carried out in this series indicates the following:

1. Variations in moisture may vary the group relations of soil microörganisms.
2. With the moisture and organic matter constant factors, a change in soil type may also change group relations.
3. The antagonistic action is shown more markedly at the lowest moisture contents.
4. *B. subtilis* and *Zygorhynchus Vuilleminii* both decompose cottonseed meal at lower moisture conditions than they do dried blood.
5. *Zygorhynchus Vuilleminii* was more active at lower percentages of moisture than was *B. subtilis*.
6. In the clay loam soil the fungus was the predominant organism, being depressed by the bacterium in its activities.
7. In the sandy loam soil the opposite proved true, i. e., the bacterium was the more active organism having its activity depressed by the fungus.

Series II

The Associative Action of B. Subtilis and Zygorhynchus Vuilleminii with Heat as the Limiting Factor

Heat as well as moisture is a factor that is of a variable nature. As the seasons come and go and even within the same seasons the changes in temperature of the atmosphere are often and varied. Necessarily these changes in the temperature of the atmosphere are followed by a change in the temperature of the soil.

These variations in the temperature of the soil are bound to exert some influence upon various groups of soil organism, some of which we know are favored by low temperatures, others by high and still other groups by intermediate temperature. The paucity of material bearing up-

on this subject has stimulated the following investigation on this very interesting phase of the soil biology problem.

Experiment XLII. This experimentation was carried out with both types of soil. The acidity was adjusted as in Series I. Cottonseed meal was used as the source of organic matter; methods, etc., were the same as previously used. The temperatures employed were 6° to 8° C., 15° to 17° C., 22° to 25° C. and 30° C. Experiment XLII was carried out with the Penn clay loam. Table XLII records the single and combined activities of *B. subtilis* and *Zygorhynchus Vuilleminii* under various degrees of heat.

TABLE XLII

THE INFLUENCE OF TEMPERATURE UPON THE SINGLE AND ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase over check	Biological Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.		
1281-1282	6-8	4.27	4.13	4.20	—.20	Bacterium
1283-1284	15-17	8.12	8.33	8.22	3.72	"
1285-1286	22-25	9.10	10.08	9.59	5.04	"
1287-1288	30	18.06	17.15	17.60	13.10	"
1289-1290	6-8	3.92	4.20	4.06	.44	Fungus
1291-1292	15-17	18.34	18.83	18.59	14.09	"
1293-1294	22-25	29.54	27.86	28.70	24.70	"
1295-1296	30	.....	34.79	34.79	30.29	"
1297-1298	6-8	3.64	4.62	4.14	—.37	Bact. and Fungus
1299-1300	15-17	23.24	23.52	23.38	18.88	"
1301-1302	22-25	31.50	22.26	26.88	22.38	"
1303-1304	30	47.04	39.41	43.32	38.72	"

It will be noticed that at the lowest temperature employed no activity was recorded, either when the organisms were acting singly or in association. A rise in temperature to 15° C. results in activating both organisms. The fungus was the more extensively influenced at this temperature, its activity being 5 times as great as that recorded for the activity of the bacterium. A further increase in the temperature is again accompanied by an accelerated activity of both organisms. The fungus is again the most influenced by the rise in temperature. The same phenomenon also holds true at a temperature of 30° C.

Considering both organisms in association we find at 15° C. that 18.88 mg. of nitrogen accumulated. This is approximately the sum of the ammonia accumulation when these two organisms are working separately. It would seem that at this temperature both organisms are working independently of each other as no antagonistic action is noted.

On raising the temperature of 22° C. the sum of the ammonia accumulated from the component activities is 6.87 mg. less than the amount accumulated from the associated activities. Judging from the fact that the activity of the fungus while acting singly was greater than this, and further that the bacterium also was active it would seem that the

bacterium was depressing the activity of the fungus at this temperature. This point is more favorably brought out if one consults figure 9, where the activity as measured by ammonia accumulation in milligrams of nitrogen has been plotted against the temperature.

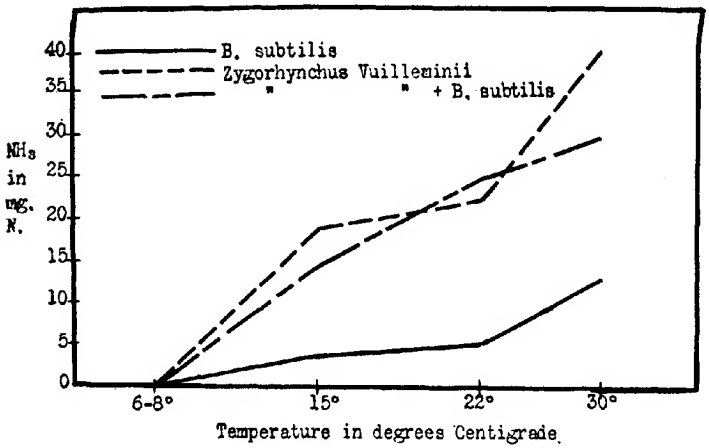

Fig. 9.—Diagram showing the influence of temperature on the associative activities of *B. subtilis* and *Zygorhynchus Vuilleminii*.

At 30° C. the phenomenon is different. The sum of the component activities would be theoretically, 43.39 mg. of nitrogen, whereas, the associative activities give us an accumulation of 38.72 mg. of nitrogen. At this temperature the activity of the fungus is equal to an accumulation of only 30 mg. of nitrogen and as the curve from 22° to 30° C. (fig. 9) tends to become highly bacterial in nature it is suggestive that the bacterium is again depressing the fungus in a larger measure than was exhibited at 22° C. The higher the temperature is raised the greater is the antagonistic action which seems to prevail.

Experiment XLIII is the same as the preceeding with a change in the type of soil. Both were inoculated at the same time so that all results are strictly comparable. As before, no activity is noted at 6° to 8° C.

In contradistinction to Experiment XLII, however, *B. subtilis* was more active in the lighter soil at 15° C. than was the fungus. This likewise holds true with regard to the respective activities in both soil types, with both sources of organic matter. The higher the temperature, the greater the activity, is the rule throughout this experiment.

When in associative action marked antagonism is to be seen. This phenomenon is present at 15° C. in this experiment, whereas at this temperature in the preceeding experiment no antagonism took place. Also,

to be noted is the fact that antagonism becomes more marked as the temperature rises in this experiment.

TABLE XLIII
THE INFLUENCE OF TEMPERATURE UPON THE ASSOCIATIVE ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase over check	Biological Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.		
1305-1306	6-8	5.04	4.40	4.72	+.22	Bacterium
1307-1308	15-17	37.58	23.45	30.51	26.01	"
1309-1310	22-25	44.87	43.75	44.31	39.81	"
1311-1312	30	55.30	54.25	54.77	50.27	"
1313-1314	6-8	4.27	4.13	4.20	—.30	Fungus
1315-1316	15-17	22.61	23.84	23.23	18.73	"
1317-1318	22-25	33.32	34.09	33.70	29.20	"
1319-1320	30	56.84	57.04	56.94	52.44	"
1321-1322	6-8	4.06	4.41	4.23	—.27	Fungus and Bact.
1323-1324	15-17	32.27	34.02	33.14	28.64	"
1325-1326	22-25	50.67	45.64	48.15	43.65	"
1327-1328	30	57.75	57.67	57.71	53.21	"

At a temperature of 15° C. the sum of the component activities in milligrams of nitrogen would be 44.74, whereas the amount actually accumu-

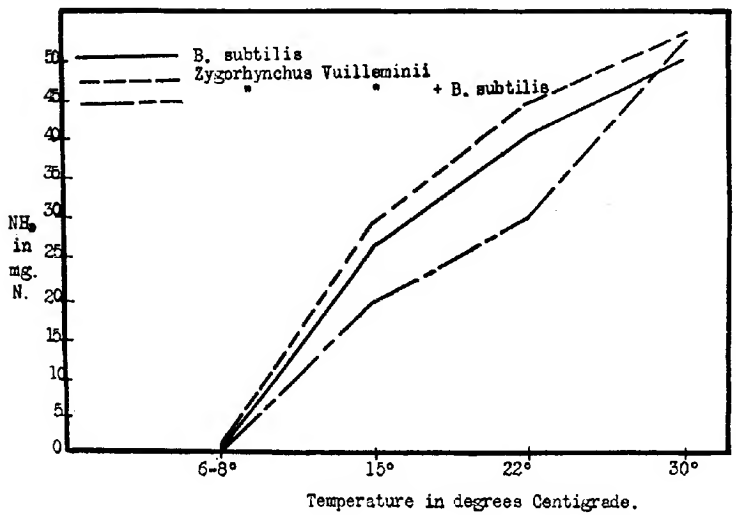

Fig. 10.—Diagram showing the influence of temperature on the associative activities of *B. subtilis* and *Zygorhynchus Vuilleminii.*

lating was only 28.64 mg., a difference of 15.10 mg. The difference at 22° C. is 25.63 mg. and at 30° C. it is 50.29 mg!

It is difficult to say whether the activity of the fungus in this type of soil has been entirely suspended by increased temperatures. It is sug-

gested from a study of the data that the activity of the bacterium becomes antagonistic to the fungus at 15° C. with the fungus recovering its depressed state at 22° C., and becoming again depressed in its activity by the bacterium at 30° C.

The activity of these organism in this type of soil is graphically represented in figure 10.

In review, the work performed seems to indicate that temperature might easily alter group relations in the soil. This alteration seems to vary with the type of soil as the activity of *B. subtilis* at 15° C. as compared with *Zygorhynchus Vuilleminii* at this temperature, either alone or in associated action, differed widely in the two types of soil employed. This also applies to the experimentation carried out at 22° C. It is further opinioned that a change in organic matter might also cause changes entirely different from the above.

Series III

The Influence of Temperature upon the Ammonification of Dried Blood and Cottonseed Meal by the Flora of a Sandy Loam Soil

Experiment XLIV. Pertinent to the above data, are some figures accumulated from another line of investigation. In an attempt to determine the influence of nitrates upon the ammonifying power of a sandy loam soil the following experiments were made.

TABLE XLIV—A

THE AMMONIFICATION OF COTTONSEED MEAL AT 22° C.

Lab. No. A	Treatment	Ammonia Accumulation			±
		Mg. N.	Mg. N.	Av. Mg. N.	Increase
51-52	$NaNO_3$ = 100 lbs. per acre.....	42.50	39.80	41.15	.60
53-54	$NaNO_3$ = 300 lbs. per acre.....	43.00	48.60	45.80	4.05
55-56	$NaNO_3$ = 500 lbs. per acre.....	47.00	48.60	47.00	5.25
57-58	$NaNO_3$ = 700 lbs. per acre.....	58.80	.....	52.25	10.50
59-60	$NaNO_3$ = 1000 lbs. per acre.....	49.10	50.15	49.62	7.87
61-62	Nothing	41.40	42.10	41 75	....

Five-pound earthenware pots were filled with a weighed quantity of soil and treated with varying amounts of $NaNO_3$ as noted in the tables below. After having left the soil in contact with the chemical for two weeks under constant moisture conditions, portions of soil equal to 100 gm. of air-dry soil were drawn from the various pots and mixed with 3-gm. charges of dried blood and cottonseed meal. Moisture allowances were made for these materials. The portions were then inoculated in tumblers for 7 days at 22° C. and at the end of that time the ammonia was determined as in the previous work. Tables XLIV-A and XLIV-B record the data. An observation of these tables shows us that dried blood was ammonified to a smaller degree than cottonseed meal, the applications of nitrate, in general, causing a lessened accumulation of ammonia

where dried blood was used when compared with the check treatment, and increasing the accumulation to a small degree where cottonseed meal was employed.

TABLE XLIV—B

THE AMMONIFICATION OF DRIED BLOOD AT 22° C.

Lab. No. A	Treatment	Ammonia Accumulation			
		Mg. N.	Mg. N.	Av.Mg.N.	Increase
63-64	$NaNO_3$ = 100 lbs. per acre.....	41.50	41.30	41.40	— 5.25
65-66	$NaNO_3$ = 300 lbs. per acre.....	36.70	36.50	36.60	—10.05
67-68	$NaNO_3$ = 500 lbs. per acre.....	44.10	44.10	44.10	— 2.55
69-70	$NaNO_3$ = 700 lbs. per acre.....	35.00	36.70	35.85	—10.80
71-72	$NaNO_3$ = 1000 lbs. per acre.....	33.40	36.10	34.75	—11.90
73-74	Nothing	47.00	46.30	46.65	.....

Experiment XLV. Upon repeating the above experiment as confirmatory work the thermostat of the incubating chamber became disarranged and induced a temperature of 15° to 17° C. for the major portion of the experiment, with the striking results shown in Table XLV-A.

Tables XLV-A and XLV-B record the experimentation carried out at 15° to 17° C.

TABLE XLV—A

THE AMMONIFICATION OF COTTONSEED MEAL AT 15° TO 17° C.

Lab. No. A	Treatment	Ammonia Accumulation			
		Mg. N.	Mg. N.	Av.Mg.N.	Increase
87-88	$NaNO_3$ = 100 lbs. per acre.....	43.90	37.00	40.45	— 8.30
89-90	$NaNO_3$ = 300 lbs. per acre.....	37.60	41.70	39.60	— 9.15
91-92	$NaNO_3$ = 500 lbs. per acre.....	33.57	28.10	30.83	—17.92
93-94	$NaNO_3$ = 700 lbs. per acre.....	33.00	32.50	32.75	—16.00
95-96	$NaNO_3$ = 1000 lbs. per acre.....	30.90	.....	30.90	—17.85
97-98	Nothing	47.70	50.30	48.75	.....

Dried blood was ammonified nearly to twice the extent as was the case in the first experiment, nitrate again causing a lessened accumulation of ammonia. Cottonseed meal on the other hand was ammonified to a smaller extent than in the previous experiment.

Also to be noted is the fact that the ammonification of cottonseed meal was not enhanced as in the previous experiment by the presence of nitrate of soda, but that the accumulation was lessened in a manner similar to the results obtained where dried blood was employed.

It has been pointed out in the preceeding pages that soil fungi seem to be relatively poor ammonifiers at low temperatures, whereas the temperature ranges for the activity of bacteria are quite wide, being somewhat lower than that exhibited by the fungi at hand. It has also been shown that fungi prefer materials of a carbohydrate nature for their activity. The following explantion of the above phenomenon suggests itself.

At 22° C. the fungi and bacteria were acting together in the degradation of the organic matter, with an antagonistic action taking place between these two groups. It is also suggested that at 22° C., in the presence of dried blood, that the bacteria were the predominant flora, whereas, in the presence of cottonseed meal the fungi could be the dominant factor. When the nitrate of soda was applied to a soil having, *a priori*, a bacterial flora, the resulting effect was to cause a decrease in the ammonia accumulation, whereas, when this chemical was applied to a flora, *a priori*, namely of a fungus nature these organisms were the most influenced.

TABLE XLV—B

THE AMMONIFICATION OF DRIED BLOOD AT 15° TO 17° C.

Lab. No. A	Treatment	Ammonia Accumulation			
		Mg. N.	Mg. N.	Av. Mg. N.	Increase
75-76	$NaNO_3$ = 100 lbs. per acre.....	77.40	.....	77.40	— 4.50
77-78	$NaNO_3$ = 300 lbs. per acre.....	76.10	77.30	76.70	— 5.20
79-80	$NaNO_3$ = 500 lbs. per acre.....	77.10	71.10	74.10	— 7.80
81-82	$NaNO_3$ = 700 lbs. per acre.....	55.80	64.70	60.25	—21.65
83-84	$NaNO_3$ = 1000 lbs. per acre.....	59.30	63.60	61.45	—20.45
85-86	Nothing	81.40	82.40	81.90	.....

At 15° to 17° C., a temperature unfavorable to the activity of soil fungi was at hand. It can be conceived, then, that the bacteria, released to a large extent from their competition with the fungi would exhibit an enhanced effect, as was manifested in Table XLV-B. The fungi being depressed by the lower temperature could not give us our maximum ammonification of cottonseed meal. The flora would then probably become bacterial in nature. Evidence of this is presented when one regards the depressing influence of the nitrate of soda, this phenomenon being in correlation with the influence of nitrate upon the ammonification of dried blood.

This phenomenon may also have a bearing upon the increased numbers of bacteria in frozen soil. Would not the bacteria be able to multiply faster when released from any serious competition, as for example the soil fungi, assuming that the latter became relatively inactive at comparatively low temperatures as the work in Part IV indicates? If this should be the case the increased multiplication at medium low temperatures would account in a large measure for the higher bacterial numbers reported in frozen soils, as the increased numbers found at medium temperatures would be present, either in an active or spore state at the very low temperatures.

General Summary.

The experimental data above submitted constitute a series of systematic researches dealing with the factors that may influence the activity of soil fungi. Several of the salient facts which have come to light during the course of the above investigations are highly suggestive and demand a word of comment with respect to their general significance.

First, with regard to the activities of the different organisms tested, it was found that the type of soil, as well as the quality of the organic matter regulated the activities of these organisms. It appears, judging from the standpoint of pure cultures that every organism will do best with a definite combination of soil and organic matter. As a general rule, vegetable matter of high quality was conducive to the greatest activity of these organisms. Dried blood was conducive to an accelerated action of two organisms.

In the second place, the experimentation concerning the activity of soil fungi as influenced by the mechanical composition of the soil indicates a wide divergency of response to increased oxygen supply. The data intimate that some fungi would be greatly benefited by an increased oxygen pressure, others but very little and still others express relation gradient between high and low oxygen pressures.

Of possible importance was the phenomenon expressed by the fungi with regard to a bettered chemical environment. The materials beneficial to one group or species may be detrimental to the other groups of organisms, suggesting a possible alteration of group relations among the microbes in the soil.

Concerning the influence of moisture upon the activity of soil fungi the results indicate that some fungi require moisture conditions that are at a variance to the requirements of other fungi. For instance, two of the forms studied demanded a dry medium for their activity, two were the most active under optimum soil moisture conditions and one required a high moisture environment for its best activity.

In the studies carried out with the influence of temperature upon the activities of these organisms a very narrow temperature range was observed, with an optimum temperature of about 30° C. for all the species studied.

In the studies carried out with the associated activities of bacteria and fungi, one finds some very interesting data, pointing to the possible changes among group relations of soil microörganisms due to food, moisture, temperature and soil conditions.

However, the data recorded must not be taken too prolixly. The limitations of the methods employed must be fully realized. Sterilized soil is somewhat different from fresh field soil. By sterilizing the soil plant-food is made available as shown by us (3) in another paper. Toxic bodies are also created as shown by Pickering (31, 32) and others (37). It is thought that the excess food offered in the form of organic matter for the ammonification process entirely alleviates any possible beneficial influences that sterilization might give to the soil by releasing plant-food.

Any toxic bodies which may have been created due to sterilization are in all probability a factor to be reckoned with. The soils used are only two of the many existing types and the fungi and bacterium tested typify

only potent leaders of two important groups of soil microörganisms. All these facts must be taken into consideration in drawing final conclusions from this work.

The results are very suggestive, however, and it is hoped that the material herein submitted will act as a stimulus for further constructive experimentation in this new branch of soil science.

In conclusion, the author's gratitude is due Dr. M. T. Cook, Prof. J. P. Helyar, Prof. A. W. Blair, and Mr. C. R. Fellers for suggestions and aid in carrying out this work, and especially to Dr. J. G. Lipman under whom the work of this thesis has been concluded.

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THE EFFECT OF SOME MANGANESE SALTS ON AMMONIFICATION AND NITRIFICATION[1]

By

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The relation of manganese to plant growth has attracted some attention in times past among plant physiologists, but it has been generally conceded that although it occurs in all plants, it does not serve directly as a nutritive element. Under artificial conditions, it has been possible to grow perfect plants in the entire absence of manganese, but applications of manganese salts have frequently been found to bring about increases in crop yields. The reasons for such beneficial effects have not been ascertained, but it has been assumed that the action was a stimulative one.

Some recent experiments by Skinner and Sullivan (6) demonstrated the fact that manganese acts in various ways as a fertilizer. It is often without influence, occasionally injurious, but usually beneficial, its effect depending apparently upon the composition and character of the soil. The oxidation in soils under treatment with manganese salts was also studied and it was found that an increase in oxidation and growth frequently occurred in aqueous extracts of poor, unproductive soils, but while oxidation was increased in fertile soils, growth was decreased, the plants showing indications of excessive oxidation. Field experiments showed practically no effect from the manganese salts, but the soil was acid, a condition which may have accounted to a considerable degree for the nature of the results.

It is suggested that when the action of manganese is beneficial, "it is probably due (1) to the increased oxidation produced in the plant roots whereby the plant is stimulated to greater activity and to increased absorption of the material useful for its growth and general metabolism; (2) *to the stimulation of the activity of microörganisms in the soil;* (3) to an increased oxidation within the soil." (6, p. 28).

The same authors also suggest that when large applications of manganese have been found to be injurious, the injury is undoubtedly due to the "excessive stimulation and excessive oxidation in microörganisms and in the plant, with a resulting change in the biochemical activities of plant and microörganisms and in the conditions of inorganic and organic soil constituents, the ultimate result of which change is injurious to the growing crop." (6 p. 29).

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It is apparent, therefore, that the effect of certain manganese salts on soil bacteria should be determined, as it may be that the effect of the compounds on the crop grown is due to the influence on soil bacteria.

Are necessary bacteriological changes encouraged or restricted by manganese salts in small amounts? What bacteriological processes are affected and how are they influenced? What relation to the effects on crop yields are the effects on certain bacteria? These are a few of the questions which arise in the minds of those who consider the bacterial side of the problem of manganese fertilization.

Kelley (2) tested ammonification and nitrification in Hawaiian soils high and low in maganese and found that nitrification took place more rapidly in the manganese soils, while ammonification was about the same in the manganese soils as in the normal soils. He attributed the greater nitrification in the manganese soils to their better physical condition and hence better and greater oxidation. The conclusion reached was that manganese does not interfere with the growth of the nitrifying or ammonifying bacteria. Later experiments (3) by the same author confirmed these results.

Montanari (5) determined the effect of some manganese salts on nitrifying bacteria and he found that 0.5 gm. of MnO_2, 0.1 gm. of $MnSO_4$ and 0.5 gm. of $MnCO_3$ in 50 gm. of soil each increased the nitrification of ammonium sulfate.

Leoncini (4) recently studied the effect of manganese oxide on nitrification and found that applications at the rate of 0.035-2.2 per cent favored nitrification, but larger amounts had no influence.

No further experiments from the bacteriological standpoint have been carried out as far as the authors are aware.

It was the purpose of the following experiments to throw some light on the problem from the bacteriological standpoint. It was planned to study the effect of certain manganese salts on ammonification and nitrification, using a normal field soil as the basis of the test.

The results obtained in the work are included in the following pages. They are necessarily somewhat preliminary in nature, dealing as they do with merely one type of soil, but the conclusions reached may be of more general application than is anticipated, and at any rate they will apply to soils of similar chemical and mechanical composition.

The Plan of the Experiment

The soil used in the tests was a Carrington clay loam, containing 0.1732 per cent of manganese as determined by the colorimetric method described by Hillebrand (1). A large sample of this soil was secured, air-dried, sieved thoroughly, mixed and stored for use.

Various series of tests were carried out using manganese sulfate, manganese chloride, manganese nitrate and manganous oxide in varying

amounts to ascertain their effect on the ammonification of dried blood and on the nitrification of ammonium sulfate.

In the ammonification tests 5 gm. of dried blood were added to 100 gm. of the air-dried soil in tumblers and thoroughly stirred in, the additions of manganese salts made as called for, the moisture content adjusted to the optimum for the soil, 12 c.c. of additional water being supplied on account of the organic matter used, and the tests incubated for 6 days. The ammonia was distilled off by the magnesium oxide method.

In the nitrification experiments 100 mg. of ammonium sulfate was added to the air-dry soil in tumblers, the manganese salts supplied, 10 c.c. of an infusion of a fresh soil added, the moisture content adjusted to the optimum, and the tests were incubated for four weeks. The moisture content was kept up during the incubation period by adding water to weight every 10 days.

All of the tests were incubated at room temperature.

Series I

The Effect of $MnCl_2$ on Ammonification

The effect of manganese chloride on the ammonification of dried blood was tested in this series, applications of 0.1 gm., 0.5 gm., 1.0 gm., 3.0 gm., and 5.0 gm. of the salt being made to duplicate portions of 100 gm. of soil. The results appear in Table I.

TABLE I

THE EFFECT OF $MnCl_2$ ON AMMONIFICATION

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	170.29	
2	Check	174.01	172.15
3	0.1 gm. $MnCl_2$	159.60	
4	0.1 gm. $MnCl_2$	156.74	158.17
5	0.5 gm. $MnCl_2$	102.17	
6	0.5 gm. $MnCl_2$	110.66	106.42
7	1.0 gm. $MnCl_2$	73.80	
8	1.0 gm. $MnCl_2$	70.12	71.96
9	3.0 gm. $MnCl_2$	25.09	
10	3.0 gm. $MnCl_2$	22.42	23.76
11	5.0 gm. $MnCl_2$	0	
12	5.0 gm. $MnCl_2$	0	0

It is apparent from an examination of this table that the chloride of manganese exerted a pronounced depressing action on the ammonification of dried blood. The depression was considerable when 0.1 gm. was used and increased rapidly with the increasing additions until with the 5.0-gm. quantity no ammonification whatever occurred.

If manganese chloride is able to bring about any increases in ammonification, it is evident that smaller amounts than those used here must be employed, at least on this soil. This result is not surprising as the smallest amount applied in this series would represent the use of 2000 pounds

per acre, which would be a very large addition. It was deemed advisable, however, to ascertain the upper limit of safety in the use of the salt from the standpoint of its effect on ammonification which represents, of course, the decomposition of organic matter. The largest amount of manganese chloride which may be applied to the soil without depressing the production of available plant-food is evidently less than 2000 pounds per acre.

SERIES II

THE EFFECT OF $MnCl_2$ ON AMMONIFICATION (CONTINUED)

This series was planned to supplement the preceeding in the effort to determine the effect of manganese chloride on ammonification. The amounts of the salt added were very much smaller than those used in the first test, being gradually increased up to the smallest amount used in that case.

The applications used and the results secured are given in Table II.

TABLE II

THE EFFECT OF $MnCl_2$ ON AMMONIFICATION—(Continued)

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	167.19	
2	Check	174.45	170.82
3	0.005 gm. $MnCl_2$	176.77	
4	0.005 gm. $MnCl_2$	170.10	173.44
5	0.01 gm. $MnCl_2$	175.51	
6	0.01 gm. $MnCl_2$	lost	175.51
7	0.03 gm. $MnCl_2$	164.68	
8	0.03 gm. $MnCl_2$	165.20	164.94
9	0.05 gm. $MnCl_2$	148.14	
10	0.05 gm. $MnCl_2$	157.62	152.88
11	0.07 gm. $MnCl_2$	151.53	
12	0.07 gm. $MnCl_2$	156.17	153.85
13	0.09 gm. $MnCl_2$	144.96	
14	0.09 gm. $MnCl_2$	151.97	148.47

On examining this table, it appears that 0.005 gm. and 0.01 gm. of $MnCl_2$ gave slight increases in ammonification, but the larger additions all depressed the amount of ammonia produced, the depression increasing with increasing additions.

There was a very slight difference in the ammonia production where the 0.05 gm. and the 0.07 gm. of the chloride were added, but the variations in the duplicate determinations would account for the results secured. It is probable that the higher result in the case of the 0.05-gm. treated soil should be considered, in which case the continuous depression in ammonification with increasing amounts of the chloride would be definitely shown. It is apparent, however, that the differences were slight and the conclusion seems warranted that increasing the amounts of the chloride increases the depression in ammonification.

The small increases produced by the two smallest applications of the chloride can hardly be considered conclusive. The differences were too small, and while the results indicate a slight beneficial effect from these amounts of manganese chloride on ammonification, it might be that the results should be interpreted to mean the absence of any effect. The nitrification results may indicate how these results should be interpreted, that is, whether the increases noted should be considered definite or not.

Applications of manganese chloride to the soil, therefore, in amounts not exceeding 200 pounds per acre of two million pounds of surface soil had no depressing action on ammonification and gave indication of a slight beneficial effect. Larger amounts of the salt, however, brought about depressions in ammonia production and these depressions gradually increased as the applications increased.

Series III

The Effect of $MnCl_2$ on Nitrification

This series was arranged to test the effect of manganese chloride on nitrification. The amounts of the manganese salt applied to the soil were the same as those used in Series I.

The treatments and results are given in Table III.

TABLE III

THE EFFECT OF $MnCl_2$ ON NITRIFICATION

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	6.250	
2	Check	4.166	5.208
3	0.1 gm. $MnCl_2$	6.250	
4	0.1 gm. $MnCl_2$	0.586	3.418
5	0.5 gm. $MnCl_2$	2.344	
6	0.5 gm. $MnCl_2$	3.125	2.735
7	1.0 gm. $MnCl_2$	0.879	
8	1.0 gm. $MnCl_2$	0.586	0.733
9	3.0 gm. $MnCl_2$	0.220	
10	3.0 gm. $MnCl_2$	0.220	0.220
11	5.0 gm. $MnCl_2$	trace	
12	5.0 gm. $MnCl_2$	trace	trace

On examining the results in the table, it becomes evident that the chloride of maganese depressed nitrification to a pronounced extent. The depression was apparent with the smallest amount, 0.1 gm., although the duplicates in this case were not entirely satisfactory, and became gradually greater until, with the largest addition of the chloride, no nitrification whatever occurred in the soil.

These results checked exactly those secured in Series I for ammonification. The depression with the smallest amount was found in both cases and with the largest addition neither ammonification nor nitrification occurred in the soil. From the results thus far it would seem that the two processes were similarly influenced by the use of maganese chloride.

It remains for the following series to determine whether the effects of the smaller applications on the two processes were the same.

SERIES IV

THE EFFECT OF $MnCl_2$ ON NITRIFICATION (CONTINUED)

This series was a continuation of the preceeding, but smaller additions of the manganese chloride were used. The amounts applied were the same as those used in Series II.

On turning to Table IV which gives the applications and the results secured, it is found that conclusions are difficult to reach. The variations among the average results were less than the differences among the duplicates in many cases and definite conclusions should not be drawn. It seems that all the amounts of the salt used here increased slightly the nitrifying power of the soil. If it is deemed, therefore, that the differences were large enough to permit of such a conclusion, it would be evident that the use of 0.09 gm. of manganese chloride would not only be safe, but would actually increase slightly the nitrifying power of the soil. On the other hand, if the results are not considered as showing any increases, at least they show no depressions and it would seem that any of the amounts used would not be injurious to the nitrifying organisms.

TABLE IV

THE EFFECT OF $MnCl_2$ ON NITRIFICATION—(Continued)

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	13.16	
2	Check	12.50	12.83
3	0.005 gm. $MnCl_2$	13.88	
4	0.005 gm. $MnCl_2$	15.44	14.66
5	0.01 gm. $MnCl_2$	12.50	
6	0.01 gm. $MnCl_2$	15.24	13.87
7	0.03 gm. $MnCl_2$	13.16	
8	0.03 gm. $MnCl_2$	13.84	13.50
9	0.05 gm. $MnCl_2$	13 84	
10	0.05 gm. $MnCl_2$	13.16	13.50
11	0.07 gm. $MnCl_2$	15.44	
12	0.07 gm. $MnCl_2$	13.84	14.64
13	0.09 gm. $MnCl_2$	13.84	
14	0.09 gm. $MnCl_2$	15.44	14.64

On comparing these results with those in the ammonification tests, it is found that there was not perfect agreement. In the case of ammonification, the use of amounts of the chloride greater than 0.01 gm. or 200 pounds per acre depressed the process, but the nitrification tests seem to indicate that much larger amounts may be used with no danger of injurious effect on the process.

It seems that the results of the ammonification tests should be considered more conclusive than those of the nitrification experiments. In the first place, they were more definite, a fact which indicates that the method used is more suitable, while in the nitrification results, the variations in

duplicate determinations were as large as differences between variously treated soils. This would suggest the possibility that the method for determining the nitrifying power of soils does not permit of perfect differentiation among soils differently treated. Perhaps a longer incubation period might have modified the results to the best extent, or some other slight change in the method may be advisable. The present results, however, must be interpreted as they stand.

It is customarily believed that the nitrifying organisms are more sensitive than the ammonifiers to an excess of salts in the soils, as well as to other unusual influences. This is due to the fact that the ammonifying group of organisms includes such a large number of bacteria of various characteristics and especially those with the ability to form spores. Furthermore, many influences which restrict the process of nitrification have been found to have little or no effect on ammonification.

Again, the fact that ammonification must precede nitrification, that the latter process can occur only following the former, precludes the possibility of the former's being depressed and the latter's being increased at the same time, at least in the case of normal soils. Under ordinary conditions ammonia does not accumulate in soils, and hence when a soil is subjected to treatment, unless ammonia production is increased, it is impossible for nitrate production to be increased. While, therefore, special treatments may restrict nitrification and not ammonification, in which case ammonia would accumulate in the soil, it is difficult to understand how the latter process could be depressed and not nitrification also.

In short, it appears that the conclusions from this work should be based on the ammonification tests and on the nitrification tests in so far as they are in agreement, and the apparent discrepancies in the latter results should be attributed to the method employed.

Manganese chloride, therefore, in amounts greater than 200 pounds per acre depressed ammonification, the depression increasing with the additions until a point was reached at which ammonification ceased. In amounts smaller than 200 pounds per acre, manganese chloride gave slight increases in ammonification, but these were not great enough to be conclusive, and the results should probably be interpreted as showing a lack of effect of the applications. With nitrification, amounts of the chloride greater than 2000 pounds per acre depressed the process, the depression increasing as in the case of ammonification until a point was reached where the process stopped. Smaller amounts of the manganese chloride, however, gave quite definite increases in nitrification, the application of 100 pounds giving the greatest effect.

It is probable, therefore, that the small increase noted in the ammonification results should be considered definite and the use of manganese chloride at the rate of 100 pounds per acre as bringing about an increase in the bacterial processes of nitrification and ammonification.

If therefore, such an amount of the chloride applied to the soil increases plant growth, the effect may be attributed partly to the influence on ammonification and nitrification.

In amounts greater than 200 pounds per acre, the chloride depressed the activities of the ammonifiers and nitrifiers and an injurious action of a manganese fertilizer might, therefore, be due to its effect on these organisms.

SERIES V

THE EFFECT OF $MnSO_4$ ON AMMONIFICATION

This series was planned to test the effect of manganese sulfate on ammonification. The applications made to the soil and the results secured are given in Table V.

TABLE V

THE EFFECT OF $MnSO_4$ ON AMMONIFICATION

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	265.28	
2	Check	262.83	264.06
3	0.1 gm. $MnSO_4$	256.90	
4	0.1 gm. $MnSO_4$	261.44	259.17
5	0.5 gm. $MnSO_4$	240.61	
6	0.5 gm. $MnSO_4$	258.41	249.51
7	1.0 gm. $MnSO_4$	218.39	
8	1.0 gm. $MnSO_4$	213.62	216.01

The use of the manganese sulfate seems to have depressed ammonification to a considerable extent, the larger amounts applied bringing about greater depressions. Only three applications of the sulfate were made here, 0.1 gm., 0.5 gm., and 1.0 gm., while in the case of the chloride, 3.0-gm. and 5.0-gm. amounts also were used, so that no complete comparisons can be made. It seems, however, that the sulfate did not bring about as pronounced a depression as the chloride did.

It is evident from this test that applications of manganese sulfate in amounts greater than 2000 pounds per acre depressed ammonification, and if any stimulation of the process is produced by the sulfate, it must be with a smaller amount than any used here.

SERIES VI

THE EFFECT OF $MnSO_4$ ON AMMONIFICATION (CONTINUED)

In order to test the effect on ammonification of smaller amounts of manganese sulfate than those used in the preceding series, a further test was arranged.

The amounts of sulfate added and the results secured appear in Table VI.

It is evident from an examination of this table that all the applications of the sulfate increased to some extent the ammonifying power of the

soil. The greatest increase, however, occurred with the addition of 0.005 gm. With the 0.01, 0.03, 0.05, 0.07 and 0.09-gm. quantities almost identical amounts of ammonia were secured, the differences which were evidenced being attributable entirely to unavoidable variations in the determinations. The amounts produced, however, were less than that obtained where the 0.005-gm. quantity was used.

It appears, therefore, from these results that the application of 100 pounds of manganese sulfate per acre brought about a stimulation of ammonification which, while not large, was still quite definite. Increasing the application up to 2000 pounds per acre occasioned a smaller increase in ammonification but still a definite gain was evidenced. Beyond that point, however, the sulfate depressed the ammonifying power of the soil, the depression increasing with increasing applications.

TABLE VI

THE EFFECT OF $MnSO_4$ ON AMMONIFICATION—(Continued)

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	166.47	
2	Check	162.80	164.64
3	0.005 gm. $MnSO_4$	180.95	
4	0.005 gm. $MnSO_4$	188.48	184.72
5	0.01 gm. $MnSO_4$	168.44	
6	0.01 gm. $MnSO_4$	184.24	176.34
7	0.03 gm. $MnSO_4$	185.18	
8	0.03 gm. $MnSO_4$	164.59	174.89
9	0.05 gm. $MnSO_4$	169.10	
10	0.05 gm. $MnSO_4$	176.44	172.77
11	0.07 gm. $MnSO_4$	172.49	
12	0.07 gm. $MnSO_4$	184.90	178.70
13	0.09 gm. $MnSO_4$	174.27	
14	0.09 gm. $MnSO_4$	184.71	179.49

If manganese sulfate is applied to soils then in amounts smaller than 2000 pounds per acre, and exerts a beneficial effect on crop yields, the effect may be due, in part, at least to the acceleration of the ammonification process. If the use of 100 pounds per acre gives the best results, it may be due likewise to the fact that ammonification is accelerated to the greatest extent by that amount. On the other hand, if applications of 2000 pounds and over of manganese sulfate depress crop growth, it may be due, partly at any rate, to the depression in ammonification, indicating as it does a depression in the decomposition of organic matter and therefore in the production of available plant-food.

SERIES VII

THE EFFECT OF $MnSO_4$ ON NITRIFICATION

The effect of manganese sulfate on nitrification was tested in this series. The amounts of the sulfate used were the same as in the first ammonification test, Series V, except that two additional amounts, 3.0 gm. and 5.0 gm. were used.

On turning to Table VII which gives the results of the tests, it is found that the sulfate depressed nitrification, the depression, with one exception, increasing with increasing additions of the salt. In one case, a larger amount of the sulfate seemed to give a smaller depression, but the difference was not great and no duplicate determination was secured. With the 3.0 and 5.0-gm. quantities, the same amounts of nitrates were secured, but the production was very small. Evidently these applications of the sulfate led to a minimum nitrification.

These results agreed quite satisfactorily with the ammonification results as far as they were comparable, the addition of 0.1 gm. depressing slightly both nitrification and ammonification, while the larger amounts gave large depressions, the extent of the depression depending on the size of the application.

TABLE VII

THE EFFECT OF $MnSO_4$ ON NITRIFICATION

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	22.38	
2	Check	30.22	26.30
3	0.1 gm. $MnSO_4$	21.06	
4	0.1 gm. $MnSO_4$	21.06	21.06
5	0.5 gm. $MnSO_4$	4.24	
6	0.5 gm. $MnSO_4$	6.64	5.44
7	1.0 gm. $MnSO_4$	6.64	
8	1.0 gm. $MnSO_4$	[1]12.96	6.64
9	3.0 gm. $MnSO_4$	2.76	
10	3.0 gm. $MnSO_4$	4.24	3.50
11	5.0 gm. $MnSO_4$	2.76	
12	5.0 gm. $MnSO_4$	4.24	3.50

[1] Omitted from the average.

Two thousand pounds of manganese sulfate, therefore, if applied to the soil may bring about a depression in crop growth because of a decrease in ammonification and in nitrification.

SERIES VIII

THE EFFECT OF $MnSO_4$ ON NITRIFICATION (CONTINUED)

The previous series showed the effect of manganese sulfate applied to the soil in amounts of 2000 pounds per acre and above. It remains to be determined whether smaller applications would accelerate nitrification and up to what point additions of the sulfate might be made with safety as regards its effect on nitrification.

The amounts of sulfate added and the results obtained are given in Table VIII. It is apparent from an examination of this table that the 0.005-gm. quantity of the sulfate brought about the greatest increase in nitrification. The amounts larger than this apparently had little effect, slight increases being noted, however, in most cases. The differences, however, were too slight to be conclusive, the variation between duplicate

determinations being as great as that between the treated and check soils. It seems evident that 100 pounds of manganese sulfate per acre had the greatest effect on nitrification and larger quantities had less influence. A noticeable depression, however, did not occur until 2000 pounds per acre were used.

These results check very satisfactorily the ammonification results. In that case also, the 100-pound application brought about the greatest increase in bacterial action, and larger amounts depressed the action.

TABLE VIII

THE EFFECT OF $MnSO_4$ ON NITRIFICATION—(Continued)

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	15.22	
2	Check	16.02	15.62
3	0.005 gm. $MnSO_4$	16.90	
4	0.005 gm. $MnSO_4$	16.86	16.88
5	0.01 gm. $MnSO_4$	16.02	
6	0.01 gm. $MnSO_4$	15.22	15.62
7	0.03 gm. $MnSO_4$	15.22	
8	0.03 gm. $MnSO_4$	16.90	16.06
9	0.05 gm. $MnSO_4$	14.44	
10	0.05 gm. $MnSO_4$	16.02	15.23
11	0.07 gm. $MnSO_4$	16.00	
12	0.07 gm. $MnSO_4$	15.20	15.60
13	0.09 gm. $MnSO_4$	15.20	
14	0.09 gm. $MnSO_4$	16.00	15.60

These studies of the effects of manganese sulfate on ammonification and nitrification as a whole show quite distinctly that if the salt when applied to the soil at the rate of 100 pounds per acre increases the crop yield, the effect may be due in part at least to the increase in the ammonifying and nitrifying powers of the soil. With larger applications than that mentioned, up to 2000 pounds per acre, increases in plant growth may be due to gains in ammonification. The gains in nitrification were quite small or entirely absent. No depressions were observed, however. Whether gains in ammonification without similar increases in nitrification are of great importance is a moot question. There is considerable evidence in support of the belief that plants will use ammonium compounds as their nitrogen food, particularly if nitrates are lacking.

It seems probable, therefore, that when the ammonification process is increased and nitrification is not, plants may use the ammonium compounds which are formed and increased plant growth may result.

When the application of manganese sulfate in amounts greater than 2000 pounds per acre is made, depressions in crop yields would be due in part to decreases in ammonification and in nitrification and consequent deficiencies in plant-food available for the crop grown.

Manganese sulfate and manganese chloride seem to act very similarly on ammonification and on nitrification. In amounts greater than 100

pounds per acre depressions in both processes occurred, while when applied at the rate of 100 pounds per acre more or less definite gains in the activities of both groups of organisms were found.

SERIES IX

THE EFFECT OF $Mn(NO_3)_2$ ON AMMONIFICATION

Manganese nitrate was the next salt tested for its effect on ammonification and on nitrification and this series was arranged to show the effect on the former process.

Table IX contains the results secured and also shows the amounts of the nitrate applied. Only three quantities were used here, just as in the case of the sulfate tests, but the depression in ammonification was so definitely shown that greater amounts were unnecessary. With the addition of 0.1 gm. of the nitrate, a slight gain in ammonification was given, but the duplicate results did not check exactly and if the low result instead of the average of the low and the high were chosen, a slight depression in the ammonifying power of the soil would be shown. It is apparent, therefore, that this result should not be considered definite and the subsequent ammonification tests and the nitrification results may indicate which interpretation should be put on these results.

TABLE IX

THE EFFECT OF $Mn(NO_3)_2$ ON AMMONIFICATION

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	265.28	
2	Check	262.83	264.06
3	[1]0.1 gm. $Mn(NO_3)_2$	278.19	
4	0.1 gm. $Mn(NO_3)_2$	259.81	269.00
5	0.5 gm. $Mn(NO_3)_2$	193.49	
6	0.5 gm. $Mn(NO_3)_2$	169.87	181.68
7	1.0 gm. $Mn(NO_3)_2$	132.29	
8	1.0 gm. $Mn(NO_3)_2$	129.85	131.07

[1] Application made on the water-free basis.

With the larger applications definite depressions were brought about, the depression increasing with the size of the application.

SERIES X

THE EFFECT OF $Mn(NO_3)_2$ ON AMMONIFICATION (CONTINUED)

In order to test the effect of smaller amounts of manganese nitrate than those used in the preceeding test, on ammonification, this series was arranged. The amounts used were different in some cases from those of the chloride and sulfate used in the preceeding series, due to preliminary observations from the previous series, but comparisons are, nevertheless, possible in several instances.

On examining Table X, which gives the results, it appears that the smallest amount of the nitrate, 0.025 gm. gave a slight increase in ammonification but the results were again not definite, the duplicate determinations not agreeing satisfactorily. If the low result be chosen as the most accurate, a depression from the nitrate appeared while the average results showed a gain in ammonification. The nitrification results may throw some light on this point, but the present result was inconclusive.

With larger applications of the manganese nitrate, however, definite depressions in ammonification were given, the depression increasing with increasing additions, up to 0.50 gm. It is evident, therefore, that in the previous series the use of 0.1 gm. of the nitrate must have given a depression in ammonification, that is, the low determination should have been considered, rather than the average of the duplicates, which did not agree as has been mentioned. The amounts of ammonia produced here were not so great and the differences appeared much more distinctly largely on that account.

TABLE X

THE EFFECT OF $Mn(NO_3)_2$ ON AMMONIFICATION—(Continued)

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	166.96	
2	Check	169.68	168.32
3	[1]0.025 gm. $Mn(NO_3)_2$	161.14	
4	0.025 gm. $Mn(NO_3)_2$	188.63	174.89
5	0.05 gm. $Mn(NO_3)_2$	166.03	
6	0.05 gm. $Mn(NO_3)_2$	[2]149.09	166.03
7	0.10 gm. $Mn(NO_3)_2$	168.46	
8	0.10 gm. $Mn(NO_3)_2$	157.14	162.80
9	0.20 gm. $Mn(NO_3)_2$	146.52	
10	0.20 gm. $Mn(NO_3)_2$	147.83	147.18
11	0.35 gm. $Mn(NO_3)_2$	124.57	
12	0.35 gm. $Mn(NO_3)_2$	117.81	121.19
13	0.50 gm. $Mn(NO_3)_2$	109.28	
14	0.50 gm. $Mn(NO_3)_2$	103.37	106.33

[1] Applications made on the water-free basis. [2] Omitted from the average.

The use of manganese nitrate on this soil apparently depressed ammonification even when used in small amounts. There was some question about the effect of the use of the nitrate at the rate of 500 pounds per acre but with larger applications the depressions were very definite. The nitrification results may indicate whether a depression from the 500-pound application should be accepted as the case or whether a slight increase should be concluded as occurring.

If, therefore, manganese nitrate is applied to the soil at the rate of 500 pounds per acre and an increase in crop yield is secured, this is probably not brought about by an effect on ammonification. If that or larger amounts of the salt when added to the soil depress crop growth, that depression may be partly at least a result of decreased ammonification.

SERIES XI

THE EFFECT OF $Mn(NO_3)_2$ ON NITRIFICATION

This series was arranged to test the effect of manganese nitrate on nitrification. The results of the test as well as the arrangement appear in Table XI. It was necessary, of course, to calculate the nitrate recovered from the ammonium sulfate nitrified, and subtract the nitrate added as manganese nitrate in order to ascertain the actual nitrification occurring in the soil. The manganese nitrate was added in solution in amounts sufficient to make an application of water-free nitrate in the amounts given.

On examining the results in the table, it appears that all the applications of manganese nitrate depressed the nitrifying power of the soil, for in no case was the entire amount of nitrate added as the manganese nitrate recovered. The lack of recovery of the nitrate added may be interpreted as showing the depression in nitrification. In other words, when 4.32 mg. of nitrogen as nitrate is given as the amount of nitrate not recovered, that amount represents the actual depression in nitrification expressed in milligrams of nitrogen.

TABLE XI

THE EFFECT OF $Mn(NO_3)_2$ ON NITRIFICATION

No.	Treatment	Nitrate Mg. N.	Average Mg. N.	Mg. N. as NO_3 recovered	Mg. N. as NO_3 added	Mg. N. not recovered
1	Check	16.87				
2	Check	12.66	14.76	.....	.....	.....
3	[1]0.1 gm. $Mn(NO_3)_2$	28.12				
4	0.1 gm. $Mn(NO_3)_2$	24.11	26.11	11.35	15.66	4.32
5	0.5 gm. $Mn(NO_3)_2$	94.92				
6	0.5 gm. $Mn(NO_3)_2$	74.92	85.42	70.66	78.30	7.64
7	1.0 gm. $Mn(NO_3)_2$	94.92				
8	1.0 gm. $Mn(NO_3)_2$	98.88	96.90	82.14	156.60	74.46

[1] Applications made on the water-free basis.

It is evident, therefore, that the larger amounts of the manganese nitrate caused greater depressions in nitrification than the smaller amount. Of course, there was probably some assimilation of the nitrate added and it is impossible to ascertain the relative importance of assimilation and nitrification. The result obtained with the addition of 1.0 gm. of the nitrate indicates that assimilation did not occur to a very great extent, for the lack of recovery was almost equal to the total nitrification. If it did play a part here, its importance was probably not great.

These results show that the application of manganese nitrate at the rate of 2000 pounds per acre depressed nitrification and larger applications brought about greater depressions.

The ammonification results were checked by these for nitrification and it would seem that the doubt in the former results regarding the effect of the smallest application should be removed and a slight depression should be considered as occurring.

SERIES XII

THE EFFECT OF $Mn(NO_3)_2$ ON NITRIFICATION (CONTINUED)

This series is a continuation of the preceeding, the amounts of manganese nitrate used being smaller than those employed in that case. The same quantities as were used in Series X in the ammonification tests were employed here.

TABLE XII

THE EFFECT OF $Mn(NO_3)_2$ ON NITRIFICATION—(Continued)

No.	Treatment	Nitrate Mg. N.	Average Mg. N.	Mg. N. as NO_3 recovered	Mg. N. as NO_3 added	Mg. N. not recovered
1	Check	22.22				
2	Check	20.78	21.50	.....	.....	.....
3	[1]0.025 gm. $Mn(NO_3)_2$	22.88				
4	0.025 gm. $Mn(NO_3)_2$	22.22	22.50	1.00	3.92	2.92
5	0.05 gm. $Mn(NO_3)_2$	19.70				
6	0.05 gm. $Mn(NO_3)_2$	20.00	19.85	—1.65	7.83	9.48
7	0.10 gm. $Mn(NO_3)_2$	24.68				
8	0.10 gm. $Mn(NO_3)_2$	26.66	25.67	4.17	15.66	11.49
9	0.20 gm. $Mn(NO_3)_2$	28.88				
10	0.20 gm. $Mn(NO_3)_2$	40.64	34.76	13.26	31.32	18.06
11	0.35 gm. $Mn(NO_3)_2$	[2]83.00				
12	0.35 gm. $Mn(NO_3)_2$	53.96	53.96	32.46	54.81	22.35
13	0.50 gm. $Mn(NO_3)_2$	60.72				
14	0.50 gm. $Mn(NO_3)_2$	53.96	57.34	35.84	78.30	42.56

[1] Applications made on the water-free basis. [2] Omitted from the average.

Turning to Table XII which gives the results as well as the arrangement of the test, it is apparent that the smallest amount of the nitrate, 0.025 gm., brought about a depression in nitrification. The larger amounts of the salt brought about greater depressions the extent of the depression varying directly with the size of the application. The results obtained in the previous series were thus checked by those secured here. The application of manganese nitrate even in small amounts depressed nitrification to a considerable extent. The smallest amount used here was 500 pounds per acre. Larger applications depressed the nitrifying action still more extensively, in some cases very little nitrification having occurred.

Assimilation may have played a part in these experiments also but how extensive it may have been under the particular experimental conditions is difficult to ascertain.

These nitrification results served to check and emphasize the ammonification results. The depression here with the smallest application of the

nitrate led to the conclusion that the depression in the case of the ammonification results was the action which should be accepted as accurate. The results with the larger amounts of the nitrate compared quite completely with those where the ammonifying power was tested. Depressions increasing with increasing applications were noted in both cases.

It is evident from these results that manganese nitrate in amounts of 500 pounds per acre and above depressed ammonification and nitrification, the depression increasing with increasing applications. If this salt of manganese in any of these amounts gives a favorable effect on plant growth, it must be due to its influence on some other factors than these two tested here. It may be due to influence on some other bacteria, or to oxidative or catalytic influences as has been suggested. If other groups of bacteria are influenced they may be particular groups concerned in the destruction of some special substances.

Of course, the amounts of manganese nitrate used were larger than those of the chloride and the sulfate which were found to increase the processes of ammonification and nitrification to some extent. If smaller amounts of the nitrate had been used, perhaps increases might have occurred here. It is apparent, however, that application of 500 pounds and over depressed the transformation of nitrogen compounds and hence if such amounts were found to depress crop yields, it might be due to their restricting influence on ammonification and nitrification processes, hence restricting the production of available plant-food.

Series XIII

The Effect of MnO on Ammonification

The influence of manganous oxide on ammonification was tested in this series. The amounts of the oxide employed were the same as those used in the case of the other salts tested. The results of the tests appear in Table XIII.

On examining this table it is apparent that all the applications depressed the ammonifying power of the soil. The depression with the 0.1 gm. of MnO was very pronounced but the larger applications did not increase the depression. The 5-gm. quantity of the oxide depressed the process about the same as the 0.1-gm. quantity. Some variations in the results were found, due to non-agreement of duplicate determinations and hence it was impossible to draw definite conclusions regarding the relative effect of the various applications. It is clearly evident, however, that the oxide in all amounts used depressed considerably the ammonifying power of the soil.

If manganous oxide is applied to the soil at the rate of 2000 pounds or over and shows a beneficial effect, it must be due to some other influence than that on ammonification. If, on the other hand, the application shows a depression, the depressing effect on ammonification might account for the effect.

Smaller amounts of the manganous oxide were not tested so that it is impossible to compare the results with this compound with those secured with the other manganese salts.

TABLE XIII

THE EFFECT OF MnO ON AMMONIFICATION

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	463.18	
2	Check	473.10	468.14
3	0.1 gm. MnO	441.40	
4	0.1 gm. MnO	457.94	449.67
5	0.5 gm. NnO	467.04	
6	0.5 gm. MnO	447.74	457.39
7	1.0 gm. MnO	466.48	
8	1.0 gm. MnO	421.25	443.87
9	3.0 gm. MnO	437.26	
10	3.0 gm. MnO	444.65	440.96
11	5.0 gm. MnO	443.33	
12	5.0 gm. MnO	452.70	448.02

Smaller amounts may have brought about slight increases as they did in the case of manganese chloride and manganese sulfate, but this may only be considered a possibility.

SERIES XIV

THE EFFECT OF MnO ON NITRIFICATION

In order to test the effect of manganous oxide on nitrification, this series was arranged. The amounts of the oxide applied were the same as those used in the preceeding series.

The arrangement of the tests and the results are given in Table XIV.

TABLE XIV

THE EFFECT OF MnO ON NITRIFICATION

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	27.77	
2	Check	20.83	24.30
3	0.1 gm. MnO	8.00	
4	0.1 gm. MnO	4.00	6.00
5	0.5 gm. MnO	2.96	
6	0.5 gm. MnO	7.11	5.04
7	1.0 gm. MnO	2.50	
8	1.0 gm. MnO	8.00	5.25
9	3.0 gm. MnO	20.00	
10	3.0 gm. MnO	16.00	18.00
11	5.0 gm. MnO	2.00	
12	5.0 gm. MnO	2.47	2.24

On examining the table, it becomes evident that all the applications of manganous oxide depressed nitrification. With one exception the depressions were very large. In the case of the 3.0-gm. addition, only a very slight depression was found, but inasmuch as the applications of 1.0 gm. and of 5.0 gm. both depressed the process to a large extent, it seems prob-

able that this result was due to some error in arranging the series or perhaps to some accidental factor interfering in the results. It appears, therefore, that the conclusion would be justified that manganous oxide depressed nitrification in all applications, the greatest depression occurring with the largest application.

These results confirm the previous ammonification tests and show that manganous oxide when applied to the soil in amounts equal to or greater than 2000 pounds per acre depressed both ammonification and nitrification, the depression increasing slightly with the amount of the oxide applied, but almost as large a depression occurred with the use of 2000 pound per acre as with larger amounts.

If manganous oxide, when applied to the soil, depresses crop growth it may evidently be due to a decrease in the bacterial activities involved in the processes of ammonification and nitrification.

Smaller amounts of manganous oxide might increase bacterial action but this point was not tested in this work.

Conclusions

These experiments on the effect of certain manganese salts on ammonification and nitrification lead, therefore, to the following conclusions:

1. Manganese chloride in applications greater than 2000 pounds per acre depressed both ammonification and nitrification, the depression increasing as the size of the application was increased, until a point was reached at which both processes ceased.

2. With smaller amounts of the chloride the effects on the two processes were not identical but tended in the same direction. Thus the applications of 100 and 200 pounds per acre gave increases which were slight in the case of ammonification but quite distinct in the case of nitrification. With amounts greater than 200 pounds per acre and less than 2000 pounds however, ammonification was depressed while no appreciable depression was apparent on nitrification.

3. Manganese sulfate when applied to the soil at the rate of 100 pounds per acre increased appreciably both ammonification and nitrification.

4. In amounts greater than 100 pounds per acre and less than 2000 pounds, ammonification was increased but to a smaller extent than with the 100-pound application, but with nitrification no gains or depressions were found with these amounts.

5. In applications equal to or greater than 2000 pounds per acre, both nitrification and ammonification were depressed by manganese sulfate, the depression increasing with the size of the application.

6. Manganese nitrate added to the soil at the rate of 500 pounds per acre or in greater amounts depressed both ammonification and nitrifica-

tion, the depression increasing as the size of the application was increased.

7. Manganous oxide when applied to the soil at the rate of 2000 pounds per acre or in larger quantities depressed both ammonification and nitrification, the depression becoming greater as the size of the addition was increased.

8. If manganese salts in small quantities increase crop yields on a soil, that increase may be due in part at least to a beneficial effect on ammonification and nitrification with a consequently greater production of avaliable plant-food.

9. On the other hand, if manganese salts when applied to the soil restrict crop growth, that restriction may be due in part to a depression of bacterial activity.

10. The amounts of various manganese salts which may be applied to any one soil without danger of depressing ammonification and nitrification are exceedingly variable.

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PRELIMINARY INVESTIGATIONS IN COMPARISON OF FIELD WITH LABORATORY EXPERIMENTS IN SOIL BIOLOGY

By

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The ultimate aim of any phase of scientific research is always its practical application. In many lines of investigation, however, methods of procedure have not yet been perfected to the extent that definite conclusions can be drawn from the results obtained.

To get the most accurate and complete results of all the factors which play a part in agricultural experimentation, the processes are first pursued on large areas in the field, then in specialized plots, then in tanks which are exposed to the out-door agencies. Further, in order to control the moisture and temperature conditions to a better advantage, vegetation experiments are carried on in the greenhouse. To combine all of these processes and in considering the ultimate constituents, the experimentation is finally completed in the laboratory.

Inasmuch as crop and fertilizer experiments are usually carried out in the field, the writer, in order to collect some data as to the influence of out-door conditions upon problems in soil biology, undertook to perform a preliminary series of experiments dealing with this subject.

A small plot 30 feet long and 9 feet wide was selected in a plowed field of the College Farm. The soil of this field was of a loam type, very nearly approaching Penn loam. A similar plot was taken in the orchard. This soil was of a Silt loam type. A third plot of the same size was staked off on a sandy soil. Hereafter, through this discussion, these soils will be designated as shale, orchard, and plot, respectively.

Each of these plots was carefully divided into 33 small areas as shown in figure 1.

These small areas, which were 6 inches square, were 18 inches apart. It was found that a section of soil 6 x 6 x 4 inches weighed 6 pounds. In each plot the small areas numbering 1 to 12 represent the ammonification series, 13 to 24 inclusive represent the areas used for the nitrification experiments, and 25 to 33 were the areas plotted off for nitrogen fixation. Area No. 1 received no treatment and the soil was not disturbed, No. 2 received dried blood, No. 3 was dug out and was put through the same

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process as in the case where additions were made. Area No. 4 received cottonseed meal. Then areas Nos. 5, 6, 7 and 8 correspond with 1, 2, 3 and 4, respectively. Likewise do areas Nos. 9, 10, 11 and 12. No. 13 was a blank with no treatment, as was No. 1. No. 14 received ammonium sulphate, No. 15 was a blank as in the case of No. 3, No. 16 received dried blood, then Nos. 17, 18, 19 and 20 and Nos. 21, 22, 23 and 24 corresponded with 13, 14, 15 and 16, respectively. No. 25 was a blank with no treatment as in the case of No. 1. No. 26 received dextrose. No. 27 received no application and got the same treatment as No. 3, then Nos. 28, 29 and 30 and 31, 32 and 33 were treated as 25, 26 and 27, respectively.

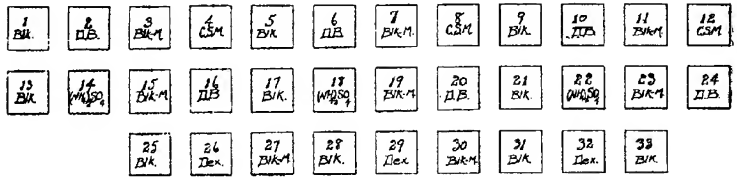

Fig. 1.—Diagram of plot containing 33 areas used in comparing field and laboratory experiments in soil biology. Areas are 6 inches square and 4 inches deep, and are placed 18 inches apart.
Blk.—Blank, unmixed; Blk. M.—Blank, mixed; D. B.—Dried blood, C. S. M.—Cottonseed meal; $(NH_4)_2 SO_4$—Ammonium Sulfate; Dex.—Dextrose.

On July 30, 1915, a series of ammonification experiments, comparing field tests with those in the laboratory were begun. By means of a hatchet, a section of soil 6 x 6 x 4 inches, weighing 6 pounds, was carefully taken out, and enough dried blood or cottonseed meal carefully mixed with it to make the proportion of 155 mg. of nitrogen for every 100 gm. of soil. In the field there were three areas for each treatment, i. e., the experiment was carried out in triplicate on each soil. Likewise, there were three blank areas which had been dug up and thoroughly "mixed" but to which nothing had been added. In all cases where the soil of the small areas was dug up, it was replaced in its original position after the mixing had been accomplished. It was then compacted so that the surface of the area was level with the surrounding soil.

At the time that the organic matter was added to the field areas, a large sample of each of these soils was taken to the laboratory and the percentage of moisture was determined by the official method. Three 100-gm. portions of each soil were carefully weighed out. To these portions enough dried blood was added so that there were 155 mg. of nitrogen to each portion of soil. Likewise, into three 100-gm. portions of each soil, cottonseed meal equivalent to 155 mg. of nitrogen was mixed;

and a third series of three portions to which no organic matter was added was also weighed out. These portions of soil were placed in tumblers and incubated for a period of 7 days at 24° to 25° C.; organic matter added to the field also had been allowed to ammonify for a period of 7 days. At the expiration of 7 days, a composite sample of 200 gm. was brought to the laboratory. Moisture determinations of the soil of each were made. One-hundred-gram portions of these field soils were then weighed out into copper flasks and the ammonia formed from the decomposition of the organic matter was distilled off by the addition of about 10 gm. of magnesium oxide. At the same time, the ammonia formed in the samples incubated in the laboratory was distilled off in like manner. The results were calculated on the oven-dry basis.

In order to get some data as to the nitrification of nitrogenous materials in the field relative with that under controlled conditions in the laboratory, ammonium sulfate and dried blood were added to small field areas, as was the cottonseed meal and dried blood in the ammonification experiments. Ammonium sulfate to the extent of 100 mg. for every 100 gm. of soil was mixed with the soils of the field plots. On the areas used to demonstrate the nitrification of dried blood, for each 100 gm. of soil a quantity of dried blood equivalent to the nitrogen in 100 mg. of ammonium sulfate was added. The nitrification experiments were also duplicated in tumblers in the laboratory. The soils were allowed to incubate for a period of 33 days, after which time the amounts of nitrates formed were determined by the official colorimetric methods.

To a third series of field areas used to demonstrate the nitrogen fixation of the soils, dextrose to the extent of 2 gm. per 100 gm. of soil was added to each 6-pound portion. After a period of 10 days' incubation, the soils were air-dried and the nitrogen determinations made. Samples of 10 gm. each were used and were titrated with N/20 acid. The other details of sampling and treatment of the field experiments, as well as the laboratory samples, were carried out as in the case of the ammonification studies.

It is seen from Table I, that while ammonification takes place quite rapidly in the field, the organic matter does not decay as rapidly as it does in the laboratory. In only one case there was more organic matter decomposed in the field than in the laboratory tests. That there was greater ammonification in the laboratory experiments than in the field might be attributed to several causes, one of which was the moisture factor due to excessive rainfall. No doubt great variation in the temperature of the soil in the field had a considerable effect upon the process. During the night, the temperature of the soil was lowered so that the rate of decay was greatly reduced, while in the laboratory, where the temperature was constant, these processes could go on without being

TABLE I

AMMONIFICATION OF DRIED BLOOD AND COTTONSEED MEAL BY THREE TYPES OF SOIL UNDER CONTROLLED CONDITIONS IN THE LABORATORY COMPARED WITH THEIR AMMONIFICATION IN THE FIELD

Kind of Soil	Determinations in the Laboratory						Determinations incubated in the Field					
	Blank Determinations mg. N.	Average mg. N.	Dried Blood mg. N.	Average minus Blank mg. N.	Cottonseed Meal mg. N.	Average minus Blank mg. N.	Blank Soil Not stirred mg. N.	Determinations Soil stirred mg. N.	Dried Blood mg. N.	Dried Blood minus Blank mg. N.	Dried Blood Average mg. N.	Cottonseed Meal mg. N.	Cottonseed Meal minus Blank mg. N.	Cottonseed Meal Average mg. N.
Shale	2.05		26.50		68.15		1.96	4.65	42.20	37.55		64.70	60.05	
Shale	1.56		30.80		68.50		2.32	3.27	44.60	41.33		57.10	53.83	
Shale	1.38	1.66	28.53	26.95	68.20	66.62	1.34	1.64	46.15	44.51	41.13	62.40	60.76	58.21
Orchard	1.92		38.10		76.20		1.61	2.66	29.25	26.59		57.28	54.62	
Orchard	1.55		37.13		73.00		1.70	1.57	28.17	26.80		58.80	57.23	
Orchard	1.38	1.62	37.10	35.82	74.95	73.10	1.78	1.46	32.12	30.66	28.01	65.78	64.32	58.72
Plot	2.46		34.80		80.78		1.06	2.34	28.20	25.86		56.75	54.41	
Plot	Lost		33.42		80.60		1.34	1.43	29.62	28.19		66.00	64.57	
Plot	2.03	2.24	35.05	32.18	78.46	77.70	1.51	1.34	23.22	21.88	25.31	66.82	65.48	61.48

influenced. Lack of aeration in the field areas is another important factor which must be considered. A third feature of importance is the excess of moisture in the field samples as is seen by the rainfall during the time that these soils were incubated.

With all the soils, in both the field and the laboratory experiments, there was a greater amount of cottonseed meal ammonified than dried blood. With one exception, twice as much cottonseed meal as dried blood was decomposed. There seemed to be very little difference in the ammonifying power of the different soils. That stirring the soil affects biological activities to a marked extent is apparent when one compares the relative amounts of ammonia distilled from the small areas which had been stirred with those which remained undisturbed.

TABLE II

THE VARIATION IN NITROGEN CONTENT OF SMALL AREAS 6 x 6 INCHES AND 18 INCHES APART

Area No.	Kind of Soil		
	Shale gm. N. in 100 gm. soil	Orchard gm. N. in 100 gm. soil	Plot gm. N. in 100 gm. soil
25	.1846	.1344	.1138
26	.1846	.1428	.1078
27	.1867	.1380	.1135
28	.1827	.1318	.1003
29	.1850	.1367	.1007
30	.1867	.1349	.0999
31	.1993	.1249	.0979
32	.2098	.1120	.0927
33	.2074	.1108	.0958

Inasmuch as there was an excessive amount of rainfall during the time that the nitrification and nitrogen fixation experiments were carried on, the field experiments with regard to moisture particularly were under conditions very different from those affecting the corresponding tests in the laboratory. The lack of nitrates in the field areas, to which ammonium sulphate had been added, as in several instances none was found, clearly demonstrated that the heavy rains either washed it away as such or as nitrates after it had been nitrified. Likewise, the nitrates resulting from the nitrification of the dried blood must have either been leached away very soon after they had been formed, or fixed at lower depths. No doubt there occurred a solution and loss of the dextrose in the nitrogen fixation experiments which were carried out in the field, as there was a heavy fall of rain during the ten days that this experiment was under observation. Due to these very abnormal conditions of moisture, the results obtained in the nitrification and nitrogen fixation tests in the field are not entered in this preliminary paper, as a comparison between field and laboratory test of these processes under such conditions would hardly be justified.

The data in Table II show an interesting fact in regard to the variation in the nitrogen content over a small area of a field, which was made plain during this experiment.

It is seen in Table II, that the nitrogen content of the extreme areas, Nos. 25 and 33, which were 15½ feet apart, in the case of the plot soil varied as much as .0180 gm. while with the orchard soil this difference was .0236 gm. Thus it is very apparent that great care must be exercised in sampling a field if a uniform sample is desired.

From the limited amount of data presented, it appears that, to some extent at least, biological experiments can be carried out in the field. Inasmuch as the moisture is one of the controlling factors, to be most successful with such experiments, a season during the year must be selected when there is apt to be least danger of heavy rainfall.

Summary

1. Biological experiments (e. g. in ammonification) can be successfully carried out in the field.

2. As a rule a greater amount of organic matter seems to be ammonified in the laboratory tests than in the field.

3. Nitrogen fixation and nitrification studies in the field are greatly interfered with by rains.

4. The nitrogen content of the soil varies considerably, even over a comparatively small area.

A STUDY OF THE ACTION OF CARBON BLACK AND SIMILAR ABSORBING MATERIALS IN SOILS[1]

By

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Carbon black is a very good agent for purifying distilled water for plant physiological purposes, its action seeming to be one of absorbing substances of a solid or gaseous nature. It is used quite generally by plant physiologists in preparing good water for culture work. Other insoluble, finally divided materials, such as ferric hydrate, aluminum hydrate, magnesium carbonate, barium carbonate and quartz flour (1, 3) are good purifiers of distilled water and extracts of soils.

Water extracts of certain unproductive soils are improved by shaking them with carbon or ferric hydrate and filtering the solution clear (2, 3). Experiments were made to ascertain whether certain poor soils would be improved by the addition of carbon and other finely divided materials direct to the soil. These experiments were made first in pots in the greenhouse, using soils which had grown the same crop repeatedly for a number of times and had become very poor. The productivity of these soils was not restored by fertilizers. Carbon black was added to and mixed with the soil in an attempt to absorb anything of a harmful nature from the soil, but the soil was not thereby improved for plant growth. In a field experiment on the Arlington Experimental Farm carbon black was added on plots growing wheat, rye, timothy, clover, corn, cowpeas, and potatoes. This experiment was conducted for six years, the same crop having been grown on the same plot each year. The carbon had no beneficial effects on any of the plots. These experiments were made by mixing the carbon with the soil. Although the carbon might have had an absorptive action in taking up substances of a harmful nature, it was nevertheless in close contact with the soil and plant roots. On this account it might be expected that no beneficial action would be shown. Similar experiments were made, in which ferric hydrate and magnesium carbonate were used, but no uniformly beneficial results were secured.

Experiments were made in similar soils by putting the carbon in porous pots, tubes, and jars and burying them in the soil. The tubes used were very porous, permitting the moisture of the soil to pass freely through the

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carbon and out again. Through the use of this method the absorbing material was not brought in contact with the soil itself or with the plant roots, but was able to absorb materials from the soil solution. If the soil contained soluble, harmful organic substances they would to a certain extent be absorbed and removed from the solution. Experiments bearing on this problem were made and are presented in the following pages. The carbon black used throughout this investigation is made by burning natural gas and collecting the carbon on cooled cylinders. It is known as the "G Elf" brand and was secured from G. L. Cabot and Co., Boston, Mass.

Effect of Carbon Black Incased in Porous Material on Growth in Pots

Several experiments were made in pots, carbon black being placed in a smaller pot of porous earthenware material and then buried in the soil in the larger pot in which the plants grew. The carbon black used in these experiments was thoroughly washed and packed in the small pot in a moist condition. The pots were the ordinary unglazed flower pots used in general greenhouse work. On account of the porous character of the pots water added to the soil during the experiment could circulate easily through the soil into the incased carbon and back again through the walls of the inner pot into the soil in which the plants were growing. The roots of the plants could in no way come in contact with the carbon black, as the top of the inner pot was covered in such a way that the soil could not mix with the carbon.

A soil was used for this first experiment which had grown cowpeas in the greenhouse, crop after crop for two years. The soil had become very poor and produced very poor cowpeas. The soil was potted in 8-inch earthenware pots and a 4-inch pot was filled with carbon and buried in the center of the 8-inch pot. In the other pot, which was to serve as a check, the small inner pot was filled with some of the same soil as the larger pot. Nine cowpea plants were planted around the circumference of the pots 1 inch from the walls. The seeds were planted March 10, 1910, and grew for 6 weeks. The growth in the two pots at an early period of the experiment is shown in Plate I, (fig. 1). Pot No. 1 is the check and No. 2 contains the carbon black. It is shown here that the growth at this early stage is better in the pot containing carbon. The green weight of the nine plants at the end of six weeks for the carbon pot was 17.8 gm. against 13.9 gm. for the check plot, an increase of 29 per cent.

A similar experiment was made growing wheat in a sandy loam soil. This soil when used in the greenhouse in pots and boxes grew good wheat. Pots of the same size as those described under the preceding experiment were used. Ten wheat plants grew in each pot and were planted around the circumference as in the case of the cowpeas. The wheat was planted

March 10 and the green weights taken April 11. The growth in the two pots was practically the same. The check pot produced 10.3 gm. green weight, while the green weight of the plants in the carbon pot was 10.4 gm.

Another experiment was made with wheat. This time a poor silty clay loam from the Arlington Experimental Farm was used. Eight-inch pots were used and the carbon, as before, was incased in a 4-inch pot and buried in the soil of the larger pot. Ten wheat plants were grown in each pot for 4 weeks. The green weight of the plants in the check pot was 3.9 gm. and that of the pots containing carbon was 6.6 gm., an increase of 70 per cent.

Still another test of this nature was made. The soil used was taken from the Smithsonian grounds and was so situated that it received the drainage and dripping from maple trees. Lawn grass in this section of the park invariably fails. Manure, lime, and commercial fertilizers have been used in attempts to secure a lawn, but with the same result, a complete failure. This ground was annually dug up and re-seeded in the early spring for several years, but the grass always failed.

The lawn soil was used in 8-inch pots as before. In one of these pots carbon incased in a small earthenware pot was buried, and in another, which was to serve as a check, the small buried pot was filled merely with the same soil used in the experiment. A mixed lawn grass seed was sown, the same amount in each pot. The grass was seeded April 12 and was cut for the first time May 11. A second cutting was made June 6, and a third June 28. The green weights of the grass are given in Table I.

TABLE I

EFFECT OF CARBON BLACK ON THE GROWTH OF GRASS IN A POOR LAWN SOIL

	First cutting gm.	Second cutting gm.	Third cutting gm.
Check	6.7	8.0	5.5
Carbon	10.6	10.0	9.0

The figures in the table show that the carbon had a very beneficial effect in this soil.

The same soil was used in a similar experiment in which clover was grown instead of grass. The check pot and carbon pot growing clover are shown in Plate I (fig. 2). The clover was planted April 12 and cut and weighed June 14. The green weight of the check pot was 90.0 gm. and that of the carbon pot 105.5 gm.

The beneficial action of carbon incased in porous material in these poor soils can be attributed only to its absorbing qualities. It would seem that the soil moisture, passing through the carbon, is robbed of its harm-

ful material, whether organic or inorganic, gaseous or liquid, and the purified soil solution passing again into the soil becomes a better medium for the growth of plants.

Effects of Carbon Black in Porous Battery Jars in Soil in Greenhouse Benches

The principle of the adsorption of harmful organic material by carbon black from poor soil was tried on a larger scale with soil on greenhouse benches. The bench used is 3 feet 6 inches wide and 8 inches deep. Partitions were placed in the bench 18 inches apart. This makes a frame 36 inches by 18 inches by 8 inches and holds approximately 250 pounds of soil. In this type of experiment, battery jars of very porous material were used. The jars are 6 inches long and 2¾ inches in diameter. They were filled and well packed with moist washed carbon, corked and buried in the soil. The jars were laid in the bed in two parallel rows, five to each row. Each row of jars was approximately 6 inches from the side of the frame and the rows were 6 inches apart. They were covered with approximately 4 inches of soil. One frame contained the jars filled with carbon. To serve as a check, the adjoining frame contained the same number of jars filled with some of the soil used in the beds.

The soil used in this experiment was taken from the flower gardens of Mount Vernon, Virginia. This soil has been under investigation by this office for several years. Some parts of the garden are producing unsatisfactory growth in spite of the fact that the soil has been well manured. Salicylic aldehyde and several other organic compounds were found in soil from certain sections of the Mount Vernon garden in former investigations and this particular sample taken for the present investigation, when subjected to the chemical process for isolating aldehydes, revealed a substance which gave the aldehyde reactions with certain chemicals, showing the presence of this class of substances in the soil (4, 5).

String beans were planted in the soil in the soil fertility greenhouse at Arlington, Va., November 1, 1915. Two rows of beans were planted in each bed, each row being over a row of tubes. The rows of beans were 6 inches apart, with 7 hills in each row. Two plants were grown in each hill, making 14 plants in each bed. The beans grew and produced fruit. They are shown in Plate II (fig. 1). The bed on the left is the check bed and contained the jars filled with soil; the bed on the right contains the jars filled with carbon. From the illustration it is seen that the carbon bed has produced the greater growth. The beans were picked and vines cut January 15, 1916, having ceased to produce fruit. The weight of the check bed was 250.0 gm. of vines and 162.5 gm. of beans in the pod and the weight of the carbon jar bed was 390.0 gm. of vines and 250.0 gm. of beans.

Other experiments of this kind were made by adding harmful organic compounds, namely, vanillin and salicylic aldehyde, to the soil in such amounts as to injure the growth. Porous jars filled with carbon were buried in the soil to determine whether or not the harmful materials would be absorbed from the soil, thereby improving the growth. Vanillin and salicylic aldehyde were selected as the organic compounds for this experiment as they have both been found to exist in some poor soils.

As in the former experiment, the bench beds were 36 inches by 18 inches by 8 inches. In one bed were placed 10 of the porous jars filled with carbon, arranged in two rows of 5 jars each, and in the adjoining bed the porous jars were filled with soil. The soil used in these beds was the silty clay loam, of moderate productivity, from the Arlington Experimental Farm. One of the vanillin treated beds contained porous jars filled with carbon and the other bed jars filled with the silty clay loam. Likewise, one of the salicylic aldehyde beds contained carbon tubes and the other soil tubes. A fifth bed having no treatment and no tubes buried in it was added as a check on the effectiveness of the aldehydes.

Fourteen bean plants grew in each bed. The seeds were planted November 1, 1915. Before planting, 5 gm. of vanillin and 3 gm. of salicylic aldehyde were added and mixed with the soil in their respective beds. Further quantities of vanillin and salicylic aldehyde were added to the surface in a water solution November 12, November 23, and December 3. The material was worked into the soil; each time 5 gm. of vanillin and 3 gm. of salicylic aldehyde were added, making a total of 20 gm. of vanillin and 12 gm. of salicylic aldehyde applied in the respective beds. This makes a total of 200 p.p.m. of vanillin for the two vanillin treated beds, and 120 p.p.m. of salicylic aldehyde for the two salicylic aldehyde treated beds. In Table II is given the yield of vines and pods from the different beds.

Considering the vanillin treated soil first, the growth in the bed which contained the carbon tubes was much better than the checkbed which contained the tubes filled with soil. Comparing the growth in both beds with that in No. 5, it is seen that the vanillin depressed the growth somewhat, but the harmful effect was overcome to a great extent by the absorption of the carbon. The growth in the two vanillin beds is shown in Plate II (fig. 2). The first bed contains the soil tubes and the second the carbon tubes.

The results with salicylic aldehyde were similar to those with vanillin. The growth in bed No. 4, which contained the carbon, was much better than that in the check bed No. 3. By comparing the growth in bed No. 5 with that in No. 3 and No. 4 it is seen that the salicylic aldehyde also produced a harmful effect. This too was partly overcome by the absorption of the carbon in the tubes. In Plate III (fig. 1) are shown the two salicylic aldehyde beds. The second bed contains the carbon tubes.

A similar experiment was made by growing lettuce in soil treated with salicylic aldehyde. In this experiment one bed contained tubes filled with soil and another bed tubes filled with carbon. The lettuce plants were transplanted November 1, 24 plants of Grand Rapids Curly Leaf variety having been placed in each bed. The amounts and time of application of aldehyde were the same as those given in the experiment with string beans. The lettuce grew rather poorly in this soil. The weight of that from the carbon bed was 645.0 gm. as compared with a weight of 600.0 gm. for the lettuce from the check bed, indicating a slightly better growth in the carbon bed.

TABLE II

EFFECT OF CARBON IN POROUS JARS, ON BEANS IN SOIL TREATED WITH VANILLIN AND SALICYLIC ALDEHYDE

Bed No.	Treatment	Porous jars buried in soil, filled with	Green weight gm.	
			Vines	Pods
1	Vanalin	Soil	172.0	138.8
2	Vanillin	Carbon	225.0	194.0
3	Salicylic aldehyde..	Soil	185.0	139.5
4	Salicylic aldehyde..	Carbon	207.0	194.2
5	Untreated	(No jars)	241.0	209.8

A review of these experiments in greenhouse bench beds shows that in a garden soil in which harmful organic compounds of an aldehyde nature exist and in soil made unproductive by the addition of organic substances, such as vanillin and salicylic aldehyde, carbon incased in porous tubes buried in the soil improves its productivity, presumably by the absorption of the soluble organic substances from the soil solution by the carbon.

Effect of Absorbing Substances Incased in Porous Materials on Growth in the Field

Experiments were also made with carbon in tubes buried in plots in the field. The experiment was enlarged in this case. Several materials, all having absorbing qualities were used, namely carbon black, wood charcoal, chalk ($CaCO_3$) and magnesium carbonate. In addition to using the battery jars, specially constructed concrete tubes and very porous tile drain were used. The cement tubes were made by coating a wire gauze tube with cement, thus making a very thin layer, which is very porous and permits water to pass through freely. These tubes were 2½ inches in diameter and 3 feet long. They were filled with the absorbing material and the ends closed with corks. The other tubes consisted of unglazed earthenware tile drains, which were very porous and permitted water to pass through freely. These were 2¾ inches in diameter and 1 foot long. They were filled and corked at each end in the same manner as the con-

crete tubes. This porous tubing was buried in the plots before the ground was seeded. Trenches were dug and the tubes laid 8 inches beneath the surface and under the rows where the plants were to grow.

These experiments were made at the Arlington Experimental Farm and in soil which had been growing cowpeas for 6 successive years. These plots are unproductive and produce small yields. The soil is an acid one, having a lime requirement of approximately 2000 pounds of $CaCO_3$ per acre. It requires periodical liming to keep the soil neutral.

Carbon black. Cowpeas were grown in rows 16 feet long and 2 feet apart. Under 4 rows tubes filled with carbon were buried and adjoining them were grown 4 rows as a check, which had no tubes. Some of these tubes were of concrete, some consisted of porous tile drain, and some of battery jars. This experiment was started in the spring of 1914 and 2 years' results were secured. Cowpeas were planted in the spring and grew to maturity, when they were cut and the weight of the cured hay taken. In 1914 the weight of the four rows with carbon tubes was 9 pounds and the weight of the check rows without tubes was 8 pounds, a slight increase of growth in the carbon plot. In 1915 the carbon plot produced 18¼ pounds of cured cowpea hay and the check plot 16 pounds. Both years there was an increase in the carbon plot.

Charcoal. This test was similar to the one with carbon, just described, except that only 2 rows of tubes were used and 2 check rows that had no tubes. The porous tubes were filled with powdered wood charcoal, which was moistened and well packed. The tubes were buried in the soil in the spring of 1914, and cowpeas grown in 1914 and again in 1915. In 1914 the two rows of cowpeas in the charcoal plot produced 6 pounds of cured hay and the check plot produced 3.6 pounds, an increase of 66 per cent for the charcoal plot. In 1915 the charcoal plot produced 12 pounds of dry cowpea hay and the check plot 6.6 pounds, an increase of 80 per cent for the charcoal plot.

Chalk. In another plot chalk ($CaCO_3$) was used in the porous tubes in order to determine whether finely divided chemicals of this character would have a similar effect to those which have only an absorbing effect. Calcium carbonate is practically insoluble in water. In addition to its effect as an absorbent it could have an effect on the soil solution passing through it by neutralizing or precipitating any acids that may be present. Three rows of tubes filled with chalk were buried in the soil, as in the experiment with carbon and charcoal. Cowpeas were grown in the rows in which the tubes were buried and also in three adjoining rows that were to serve as a check. In 1914 the weight of cowpea hay for the chalk plot was 7.0 pounds, and that of the check plot was only 3¾ pounds. In 1915 the differences were not so large; the chalk plot produced 12 2/3 pounds dry weight, and the check plot 10½ pounds. The growth in the first year

of the experiment is shown in Plate III (fig. 2). The three rows on the left have the chalk tubes; the three rows on the right are without tubes.

Magnesium carbonate. Experiments were made with magnesium carbonate, which is finely divided and, aside from its absorbing qualities, would also produce a chemical reaction with the acids of the soil solution similar to that with chalk. Three rows of tubes were used, cowpeas being grown as before, and the growth was compared with that on three other rows growing beside them.

In 1914 the growth in the magnesium carbonate plot was 14 pounds and in the check plot 14¼ pounds, and in 1915 the weights for the magnesium carbonate plot was 18.0 pounds and for the check plot 17¼ pounds. The magnesium carbonate seems to have had practically no effect, for in the first year there was a reduction of one-quarter of a pound, and in the second year there was an increase of three-quarters of a pound.

The magnesium carbonate and its check plot were, however, on a different part of the farm from the plots on which the other tests were made. The plots with carbon, charcoal, chalk and their checks adjoined each other and the soil conditions were more likely to be similar, whereas the conditions in the part of the field where the magnesium carbonate test was conducted were probably very different. This is indicated by the greater yield in the plot. The effectiveness of the magnesium carbonate in this respect is therefore not ascertained by this test.

The contents of the tubes were examined chemically for absorbed or precipitated material by E. C. Lathrop of this laboratory. The carbon black and charcoal contained a small amount of a liquid fatty acid but no aldehydes could be isolated. The calcium carbonate and magnesium carbonate contained a small amount of fatty acids and showed the presence of aldehydes. The amounts in all cases were too small for further study. The fact that aldehyde reactions were obtained from the carbonates and not from the carbon and charcoal would indicate either a destruction of the aldehydes, possibly by oxidation through absorbed oxygen, or else that the aldehydes, when once absorbed, are tenaciously held by these substances.

Summary

It is pointed out that finely divided carbon is a good agent for physiologically purifying distilled water and certain poor soil extracts, and that by its absorptive qualities it improves the solution as a medium for plant growth.

The test made by mixing carbon black with poor soils failed to effect an improvement, as the carbon, even though it might have had an absorptive action, would itself be intermingled with the soil and be in contact with the plant roots.

With carbon incased in porous earthenware pots buried in soil, the growth of grass, clover and cowpeas was improved when growing in a poor unproductive soil in the greenhouse.

On benches in the greenhouse a soil which contained salicylic aldehyde and other organic compounds was improved for the growth of string beans by the absorptive action of carbon buried in porous tubes in the soil.

In an experiment with string beans and lettuce in greenhouse benches a soil made poor by the addition of salicylic aldehyde and vanillin was improved in productivity by the action of carbon incased in porous tubes.

In a two years' field experiment carbon, charcoal, and chalk, when put in porous tubes and buried in the plots, caused a good increase in growth of cowpeas.

The beneficial action of carbon and other absorbents may be attributed to its removing something from the soil solution which is harmful to plants. The soil moisture passing through the carbon in its process of moving downward and upward in the soil would be robbed of any such material. Soils which contain soluble organic substances harmful to plants would be improved for crop growth.

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PLATE I

Fig. 1.—Effect of carbon black in a poor soil on cowpeas. (No. 1 check, No. 2 carbon black buried in the soil in a porous cup).

Fig. 2.—Effect of carbon black in a poor grass soil or clover. (No. 1 check, No. 2 carbon black buried in the soil in a porous cup.)

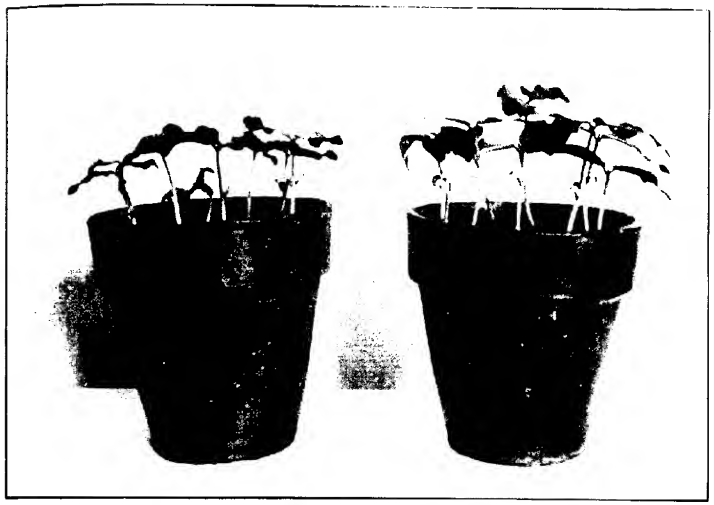

Figure 1

Figure 2

Figure 1

Figure 2

PLATE II

Fig. 1.—Effect of carbon in porous jars buried in a poor garden soil, on string beans grown on the greenhouse bench. (Bed on left contains jars filled with soil; bed on right contains jars filled with carbon black).

Fig. 2.—Effect of carbon black in porous jars in a soil to which vanillin was added, on string beans grown on the greenhouse bench. (Bed on right contains jars filled with soil; bed on left contains jars filled with carbon black).

PLATE III

Fig. 1.—Effect of carbon black in porous jars in a soil to which salicylic aldehyde was added, on string beans grown on a greenhouse bench. (Bed on left contains porous jars filled with soil; bed on right contains porous jars filled with carbon).

Fig. 2.—Effect of chalk in porous jars buried in the soil in the field, on cowpeas. (Jars filled with chalk under the three rows to the left; no chalk under the three rows on the right).

Figure 1

Figure 2